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# THE ORIGIN AND EARLY HISTORY OF THE GERM-CELLS IN SOME CHRYSOMELID BEETLES.

BY

ROBERT WILHELM HEGNER.

A Thesis

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WITH 4 PLATES.

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## I. INTRODUCTION.

The germ-cells of the Metozoa have been in recent years a favorite subject for investigators. The theory of the continuity of the germ-plasm expressed by Galton in 1872 and later (1885) elaborated by Weismann has focused the attention of embryologists upon the reproductive cells. Many remarkable discoveries have been made in the late stages of the history of these cells, but comparatively little effort has been directed toward their early embryonic development. The work which forms the basis of the present paper was undertaken in an attempt to clear up some of the problems which have resulted from a large number of disconnected studies on the embryology of the Insecta. The lineage of the germ-cells of *Calligrapha multipunctata* is described in the following pages from a preblastodermic stage until the sex of the embryo can be recognized. Two other Chrysomelid beetles, *Calligrapha lunata* and *Leptinotarsa decemlineata,* are referred to in the course of the paper, but *C. multipunctata* has received the largest share of attention.

The work was begun at the University of Chicago and was continued at the Marine Biological Laboratory at Woods Holl, Mass., and at the University of Wisconsin. I wish to thank the members of the zoölogical staffs of these institutions for their kindness and for the facilities granted to me. I am greatly indebted to Professor Wm. S. Marshall for his valuable and generous aid and the use of his extensive library; and I am also under obligations to Professor C. O. Whitman for his helpful suggestions and advice. I wish to express my gratitude to my wife for her assistance in cutting sections and in preparing the manuscript for the publishers.

## II. REVIEW OF THE LITERATURE UPON THE ORIGIN OF THE GERM-CELLS IN THE INSECTA.

### *Introduction.*

Only a few papers have been devoted exclusively to the origin and early development of the germ-cells of the Insecta. These contributions do not represent by any means our knowledge of this subject, for almost every work on general insect embryology contains observations on the primitive germ-cells, although usually in a subordinate degree.

Brandt (1878), Heymons (1891), Graber (1891) and others have provided a more or less complete historical account of this phase of embryology, but the great number of recent papers dealing with the subject makes it advisable to give the following descriptions which attempt to present, in a brief manner, the numerous fragmentary sketches of the various authors.

### 1. *Lepidoptera.*

The earliest worker whose results are worthy of consideration is Herold (1815). He has given a remarkably good description of the gross aspects of both ovaries and testes in several species of Lepidoptera, principally *Papilio brassica,* from a late embryonic period to the adult stage. For us the chief value of his results lies in his discovery that the sex of the larva is already determined before hatching. He found the ovary to consist of four tubules, and the testis of four small sacs; the former with a duct at its posterior end and the latter with a duct extending from the center of its side.

In *Bombyx pini,* according to Heymons (1891), Suckow (1828), distinguished the male from the female before the larva hatched, thus confirming Herold's results. This author described the rudiment of the germ-glands as an outgrowth from the hind-intestine "das sich späterhin durch eine Furche theilt und nach und nach vom Darmkanale abgestossen als zwei seitlich verlaufende hohle Fädchen die Geschlechtsorgane im ersten Entwurf darstellt."

The reproductive organs could not be found by Meyer (1849) in *Liparis auriflua* until the caterpillars were over three weeks old, and he wrongly pronounced the young larvæ sexless. He was the first, however, to make finer histological examinations of the developing germ-glands.

Bessels (1867) made a more accurate microscopical examination of the embryonic germ-cells than did Meyer (1849), and, contrary to the results of the latter, he found that "Die Anlage der Sexualdrüsen findet bei den Lepidopteren im Ei statt, und es wird bereits hier die Verschiedenheit des Geschlechts vollkommen deutlich." In a late embryonic stage of *Zeuzera æsculi* he found the rudimentary germ-glands at either side in the eighth abdominal segment embedded in the fat-body. They consisted of many transparent nucleated cells which separated to form the testicular follicles or ovarian tubules, about which a structureless membrane formed.

In *Tinea crinella,* Balbiani (1869-72) found that "l'organe sexuel est dèjà parfaitement perceptible à une époque oú l'embryon n'est encore représenté que par son rudiment ventral, et n'offre encore aucune trace de ses autres appareils organiques. A cette phase peu avancée de son existence, l'organe reproducteur forme une petite masse ovalaire simple, composée de minimes cellules rondes et transparentes. . . Cette masse est appliquée à la face interne de l'extremité inferieure du rudiment ventral. . . " This unpaired oval mass later divided, one-half going to either side of the body.

Brandt (1878) did not study the early development of the germ-cells but described both ovaries and testes in embryos of *Pieris brassica* just before hatching. "Die Anlagen der Genitaldrüsen selbst fanden sich im Embryo an der Rückenwandung der Leibeshöhle, etwa im achten Körpersegmente, rechts und links dicht am Herzen, dessen Peritonealüberzug auf sie überging. In ihrem jüngsten Entwicklungsstadium stellten sie je einen mehr oder weniger elliptischen Körper dar, welcher durchweg aus rundlichen Embryonalzellen mit amöboid gestalteten Kernen bestand." He found, as did Herold (1815), that the female germ-gland could be distinguished from the male by its duct which extended from the posterior end, while in the latter the duct is attached to the side.

O. and R. Hertwig (1881) found the rudiments of the germ-glands of *Zygœna minos* lying between the somatic and splanchnic layers of the mesoderm (Taf. II, Fig. 4).

According to Graber (1891), both Tichomiroff (1882) and Selvatico (1882) identified the germ-glands of *Bombyx mori* in comparatively early embryonic stages. The earliest embryo examined contained a germ-gland composed of a group of cells surrounded by mesoderm. It lay between the "Proctodæum und dem Mesenteron" in close connection "mit dem Faserblatt des Mitteldarmes."

The male germ-glands of *Zygœna filipendulœ* and other Lepidoptera were found by Spichard (1886) to arise from the mesoderm and not from a muscle fiber (Schneider, 1885). In late embryonic life each germ-gland consisted of four large "Urzellen der Geschlechtsanlage" with small cells lying between them, the whole mass being surrounded by a flat-celled epithelium. As the four "Urzellen" increased by mitosis, the surrounding sheath grew inward, thus establishing the four testicular follicles.

The earliest appearance of the primitive germ-glands of Lepidoptera was recorded by Woodworth (1889) in *Euvanessa antiopa.* About the time the blastoderm was completed, a group of cells became cut off from the ventral plate near its posterior end; these cells remained "in contact with the ventral plate at the place where they are produced. Later stages show that these cells produce the generative organs. The generative organs thus appear to be produced by an infolding of the ectoderm, or possibly of the blastoderm before the ectoderm is produced but from a position which is later to become ectoderm."

In a nine and a half day embryo of *Pieris cratægi,* Graber (1891) figured the "Anlagen der Samendrüsen" on either side of and dorsal to the intestine, thus occupying a position similar to that found by Selvatico (1882) in *Bombyx mori.* Each "Anlage" consisted of a few large cells and was surrounded by a small celled epithelium, the whole being embedded in fat-body.

Silk worm embryos, having appendages well developed, were found by Toyama (1902) to contain the rudiments of germ-glands. "Although the clusters of germ-cells are normally seen to occur in the third and sixth abdominal segments, we often observed them in all other abdominal segments with the exception of the anal; and in one case, we observed them even in the mesothoracic segment. We are thus in a position to say that the genital cells originally arise in each body segment." They differentiated from the cells of the mesoblastic somites.

The most recent account of the origin of the germ-cells in Lepidoptera was published by Schwangart (1905). He found in *Endromis versicolora* the first indication of a germ-gland two to four hours after blastoderm formation. A part of the blastoderm in the posterior region of the egg, but not at the posterior end, became several layers thick; here the inner cells were large, richer in yolk, and their nuclei had one or two nucleoli, but less chromatin than the (overlying) blastoderm cells. These inner, primitive germ-cells soon became amœboid, and, several hours before mesoderm formation, separated into groups which moved forward through the yolk. Each group divided, half of the cells migrating to either side of the body, where they lay near the cœlom in the fourth to sixth abdominal segments. Their history was not carried further.

### 2. *Diptera.*

The Diptera have received their full share of attention from insect embryologists and the works relating to species of this order are very numerous. They are also the first, and for a long time the only insects in whose eggs pole-cells were discovered; this group is, therefore, of great interest to us since pole-cells are also present in the development of the Chrysomelid beetles considered in this paper.

Robin (1862) unknowingly discovered the early segregation of germ-cells from somatic cells in the nearly transparent eggs of *Tipulides culiciformes.* Before the blastoderm was formed there appeared "par gemmation de la substance hyaline du vitellus" a number of "globules polaires" which he likened to the polar bodies of other animals. These four to eight buds each developed a nucleus which gave to it the character of a true cell. These cells were supposed to take part in the formation of the blastoderm near the spot where they were protruded. Robin did not orient the eggs correctly for he says "C'est aussi le point où apparaitre plus tard l'extremité céphalique," a statement that Weismann corrected the following year.

Although Weismann (1863) had observed these "globules polaires" in the eggs of *Chironomus nigro-viridis* and *Musca vomitoria* several years before Robin's papers appeared, he did not publish his results until 1863. Weismann noticed at the pointed posterior end of the egg four indefinite, bright spots lying in the "Keimhautblastem." These developed into four bud-like protrusions which were entirely cut off from the egg and lay in the space between the vitelline membrane and the surface of the egg. These "globules polaires" or "Polzellen," as Weismann called them, consisted of homogeneous protoplasm containing a nucleus and one or two yolk-granules. Weismann thought "dass der Modus ihrer Genese innerhalb des Begriffes von der freien Zellenbildung fällt, wie ihn die ältere histologische Schule aufgestellt hat." The four primitive pole-cells divided each into two, sometimes even before they were entirely separated from the egg; these resultant eight pole-cells became confused with the developing blastoderm-cells, and Weismann could not follow them further.

Neither Robin nor Weismann realized the importance of these pole-cells and it remained for Metschnikoff (1865) and Leuckart (1865) to announce their true significance. Leuckart (1865) described their formation in the pædogenetic larva of *Miastor* and confirmed Metschnikoff's (1865) statement that they were really the primitive germ-cells and entered into the constitution of the pseudovarium. Leuckart could distinguish the single nucleus of the primitive pole-cell when there were only eight to ten nuclei in the pseudovum.

A year later Metschnikoff (1866) gave a more detailed account of the history of the pole-cells in *Simula sp.* and *Miastor.* In *Simula* four to five pole-cells were present at the posterior end of the egg, each containing a nucleus and very fine yolk-granules. In *Miastor* the genesis of the pole-cells was traced more accurately than in *Simula.* Eggs were found containing only two nuclei which were supposed to result from the division of the germinal vesicle. These nuclei continued to give rise to others by division until twelve to fifteen were produced, one of which, lying at the pointed pole of the pseudovum, became surrounded by a thick, dark yolk mass and with it separated as a distinct membraneless cell, the first pole-cell. This then divided into two and later into four cells. These four then separated into two groups of two cells each and were recognized as the primitive reproductive organs lying in their definite positions. The two large pole-cells of each group were already divided and enclosed in an epithelial layer of embryonic cells at the time of hatching.

Grimm (1870) described in the parthenogenetic eggs of a species of *Chironomus,* a membraneless cell which divided into two, then four pole-cells. He recorded this interesting variation. "Manchmal aber theilt sich der Kern der ersten Polzelle noch in der Schicht des Bildungsdotters liegend, so dass in dem Polraume auf einmal zwei Polzellen erscheinen." The division of these pole cells into two groups, each of which became surrounded by embryonic cells and constituted the germ-glands, confirmed Metschnikoff's (1886) account.

According to Packard (1872) the eggs of *Pulex canis,* one or two hours after deposition, contained "four distinct polar cells apparently immersed in protoplasm, and a small indistinct one in addition." They were distinctly nucleated and held in place by the vitelline membrane. Thirty hours later the blastoderm was fully formed and "Soon after this the polar cells break down and disappear."

In another flea, *Pulex felis,* Balbiani (1875) found that the "Anlage" of the reproductive organs was already visible after the formation of the embryonic envelopes. It was a small naked mass of clear cells lying in the posterior inner side of the abdomen.

In a later work Weismann (1882) described events in the pole-cell development of *Chironomus sp.* which differed from those found in *C. nigro-viridis* in 1863. In *Chironomus sp.* the primitive pole-cell nucleus was thought to have probably originated from elements of the cleavage nucleus in the yolk mass and migrated to the surface of the egg; there it entered a protoplasmic process of the "Keimhautblastem" together with a number of yolk-granules which flowed in with it. This nucleus often divided, as Grimm (1870) had previously recorded, before the protrusion was entirely cut off from the egg, resulting in two pole-cells. A second bud arose in the same place as the first and passed through a similar process of separation and division. Thus four cells were produced; these increased by division to eight, and finally to twelve, which lay on the surface of the posterior pole. They did not become indistinguishable from the blastoderm-cells, as was described for *Chironomus nigro-viridis,* but "später wenn sich die Keimhaut gebildet hat, lagern sie sich deren Oberfläche locker auf, oft zu zwei Gruppen formirt." After the germ-band was formed two to four similar cells were found in many eggs outside of the embryonic membrane, but their derivation from pole-cells could not be determined.

Weismann described in one egg of *Chironomus sp.* a nucleated protrusion at the anterior end which was capable of amœboid movements and probably migrated upon the surface and was lost to view. In another case no nucleus was visible, but the anterior ball of protoplasm divided into eight bodies of different sizes, which after fifteen minutes dissolved. These were thought to be polar bodies, but might easily have been mistaken for pole-cells.

The pole-cells were accurately traced to the definitive germ-glands by Balbiani (1882, 1885) in *Chironomus.* One pole-cell usually appeared first; but, as previously observed by Grimm (1870) and Weismann (1882), sometimes two were protruded simultaneously. These two divided to form four and then eight pole-cells. Small non-nucleated protoplasmic globules were pushed out of the egg at both anterior and posterior ends, disintegrating later into a mass of granules in the polar cavities. Balbiani claimed that Robin (1862) and Weismann (1863), both of whom recorded twelve or more pole-cells, were deceived by these globules, as there should have been only eight pole-cells. These droplets would also explain the protrusion discovered by Weismann (1882) at the anterior end of the egg. Balbiani, however, did not find the anterior nucleated globules which Weismann considered polar bodies. He agreed with previous workers that the pole-cells did not arise by free formation during or after germination (Robin, 1862, Weismann, 1863), but were derivatives of the cleavage nucleus (Leuckart, 1865, Metschnikoff, 1866, Grimm, 1870). After the first division of the segmentation nucleus the anterior daughter nucleus probably gave rise to the blastodermic nuclei of that region, while the posterior furnished the two pole-cell nuclei and those of the posterior blastoderm-cells. After the eight pole-cells were formed, an elongation of the vitellus forced the group against the egg membrane causing a blastodermic depression.

In the next stage the pole-cells were found inside of the blastoderm, but Balbiani did not determine whether they passed through it, or a clear space was left in the blastoderm for their entrance. They now consisted of two separate masses, each containing two quadrinucleated cells. This was supposed to have come about in the following manner. The eight original pole-cells fused in pairs, producing four binucleated cells; then the nuclei in these four cells each divided producing four quadrinucleated cells, two of which then moved to either side of the body. No further change took place within the two germ-glands until the time of hatching. Their position was altered by the contraction of the ventral plate and, in the young larva, they lay in the ninth abdominal segment level with the union of the mid- with the hind-intestine. Each germ-gland now acquired an epithelium of flattened cells. The testes could, at the end of embryonic life, be distinguished by their narrowness, attenuated ends, and many celled constitution, from the ovary, which was broad, obtuse, and composed of only a few large cells. One of Balbiani's conclusions was that "les glandes génitales des deux sexes ont une origine absolument identique, naissant de la même substance et au même point de l'oeuf."

*Chironomus* was also studied by Jaworowski (1882). This author was led to believe that the germ-glands of insects developed from a single mother cell, which gave rise by division to daughter cells, these in turn became mother cells and produced daughter cells. In this way the "Kammern," "Endfaden" and "Ausführungsgang" were all supposed to have arisen.

Three papers record the results of Schneider's (1885) observations "über die Anlage der Geschlechtsorgane der Insecten." Although his work was done chiefly on *Chironomus plumosus, C. plumicornis,* and a viviparous Cecidomyian, nevertheless he has made the following unique generalization: "Die erste Anlage der Geschlechtsorgane der Insecten besteht, soweit ich dieselbe verfolgt habe, in einer Muskelfaser, welche sich von einem Flügelmuskel abzweigt. Sie sitzt also vorn und hinten an der Hypodermis. In der Mitte derselben entsteht eine Anhäufung von Kernen durch welche die Muskelfaser erst spindelförmig, dann eiförmig aufschwillt. Wir wollen sie als die Geschlechtsanlage bezeichnen."

Kowalevsky (1886) observed in *Musca* that the division of the cleavage nucleus into two took place near the pointed end of the egg. The nuclei produced by these two reached the posterior end first and formed pole-cells which entered the cavity between the egg and the yolk membrane. The further history of these cells was not followed.

Voeltzkow (1889) stated that in *Musca* the pole-cells pressed the blastoderm-cells inward forming a wedge which projected into the yolk. Blastoderm-cells separated from this wedge, wandered into the interior, and became the so-called yolk-nuclei. He further stated that "die Polzellen wandern mit dem Keimstreifen auf die Dorsalseite und in den Enddarm hinein."

In his work on *Calliphora,* Graber (1889) described the pole-cells, but not until the blastoderm had formed. They were found outside of the blastoderm-cells and were smaller than the latter, and stained more intensely. "Ihre Zahl ist eine ziemlich constante und beträgt ca 25-35." They were figured in a cross section (Taf. VII, Fig. 91) where they occurred in the amniotic cavity. Graber seldom saw them in *Calliphora* and never observed them in *Lucilia.*

The section method was used by Ritter (1890) in studying the development of the germ-glands in *Chironomus.* He found that the first pole-cell differentiated at the posterior end of the egg when there were a large number of nuclei scattered about in the yolk. A second pole-cell was protruded close behind the first. Each carried out of the egg part of a flat mass of protoplasmic granules, the "Keimwulst," which, in section, formed a wreath around the nucleus. The two original pole-cells increased by division to four and then to eight. Two divisions of each pole-cell nucleus now occurred, resulting in eight quadrinucleated cells; these seemed to move of their own accord through the blastoderm which closed after them. They now lay at the posterior end of the germ-band from whence they were possibly moved anteriorly by the growing forward of the entomesoderm. The mass of pole-cells finally divided into two groups which occupied a position on either side of and dorsal to the hind-intestine; there they remained until after the larva hatched, when they became the definitive sex-organs. A more detailed account of Ritter's results will be found in Part IV on the pole-disc.

The germ-glands were found by Pratt (1893) in *Melophagus ovinus* during the entire larval period. They were small paired structures lying on either side of the abdomen embedded in the fat-body.

Lowne (1890-95) did not find the pole-cells in the Blow-fly, probably, because of their small size, and similar appearance to other embryonic cells. In a 1 cm. larva he described as rudimentary germ-glands "two pairs of encapsulated groups of small embryonic cells, situated in the fifth abdominal segment embedded in the fat-bodies on either side of, and dorsal to, the alimentary canal." The pole-cells were considered by Lowne as "the segmentation spheres of the vegetative pole of the egg."

Escherich (1900) began his study of the embryonic development of *Musca vomitoria* and *Lucilia cæsar* after the formation of the blastoderm. He traced the history of the pole-cells from this time until the germ layers were completely separated. The pole-cells were first discovered as a group lying in a posterior enlargement of the ventral groove. From here they were carried around upon the dorsal side by the growth of the "Mittelplatte." The pole-cells migrated from here into the embryo through a "Polzellencanal;" they were last observed in the lumen of the "hinterer Entodermkeim" which was connected with the inner end of the proctodeal cavity.

Lecaillon (1900) has made the following general statement concerning the origin of the germ-cells in *Culex pipiens*. "L'étude du développement embryonnaire des insectes montre que, chez ces animaux, les cellules sexuelles, les *gonades,* se séparent de très bonne heure des cellules somatiques et semblent être toujours de nature ectodermique. Elles se groupent bientôt en deux petites masses pleines s'entourant chacune d'une enveloppe formée de cellules mésodermique aplaties. Souvent ces deux petites masses ne se modifient plus, au moins dans leur structure intime, et restent telles quelles jusqu' à la fin du developpement embryonnaire; ce sont les rudiments ou ebauches des organes génitaux. . . Pendant toute la durée de la vie larvaire, les organes génitaux demeurent à l'etat d'ébauche. Celle-ci consiste en deux petits massifs cellulaires situés dans la sixième anneau abdominal."

The most recent and best contribution to the genesis of the pole-cells of Diptera is that of Noack (1901). He found a dark granulated layer "Dotterplatte" at the posterior end of the egg of *Calliphora erythrocephala* similar to that discovered by Ritter (1890) in *Chironomus*. Each pole-cell took part of this layer of granules with it as it passed through the "Keimhautblastem." This was the first cell differentiation. At this time there were fifteen to twenty pole-cells present, and not one to twelve, as Weismann (1863) and others have claimed; these pole-cells divided forming a single layer. This layer became several cells thick and formed the polar mass characteristic of Dipterous eggs at this stage of development. This mass of pole-cells was then passively carried into the dorsal groove of the germ-band where it was connected with the tissue destined to become the mid-intestine as Escherich (1900) had described it in *Musca vomitoria.* The pole-cells always remained connected with the yolk; they later began to wander through the entoderm, not by means of a definite canal (Escherich, 1900), but through an indefinite gap. After this migration had taken place, the pole-cells were found lying isolated among the entoderm cells from which they were distinguished by their darker pigmentation and smaller size. Soon, however, these distinguishing features became obliterated and the further history of the pole-cells could not be followed.

### 3. *Hemiptera.*

Until recently the Hemiptera have held a position next to the Diptera regarding the early appearance of germ-cells. As we shall see later germ-cells have been found by modern methods of study at a much earlier stage in the Hymenoptera and Coleoptera than thus far reported in the Hemiptera.

Huxley (1858) was the first to study the development of the pseudovarium in *Aphis.* He thought that the reproductive organs arose from the inner layer of the two-layered blastoderm.

Metschnikoff (1866) found in the viviparous Aphid, *Aphis rosæ,* shortly after blastoderm formation, a group of cells, "Keimhügel," projecting into the central yolk mass at the posterior end of the egg. These cells could not be distinguished from those constituting the blastoderm. The anterior part of this "Keimhügel" separated from the remainder and became an oval mass, the rudiment of the reproductive organs, "Genitalhügel." This rudiment, which lay within the tail fold, was carried by the latter into the position of the definitive germ-glands. Meanwhile, the nuclei increased in number, the whole mass divided into horseshoe shaped halves which migrated to either side of the body. No "Genitalhügel" was found in *Aspidiotus nerii, Corixa* and *Psylla.* In the larva of *Psylla,* however, Metschnikoff described the germ-glands as follows: "Dicht neben den Lappen des secundären Dotters befinden sich jederseits bei den Larven und Imagines von *Psylla,* die Geschlechtsorgane—ein Umstand, welcher für meine Meinung über die Rolle des secundären Dotters als Fortpflanzungsmaterial zu sprechen scheint."

The development of both the parthenogenetic and the fertilized eggs of the Aphids was studied by Balbiani (1866-1872). In the eggs of the viviparous Aphids this author (1866) was able to see "un noyau granuleux fort pâle dans la vesicule postérieure, moins nettement dans l'antérieure, celle-ci présentent donc tous les caractères de veritables cellules. Ce sont ces vésicules ou ces cellules qui vont être l'origine des éléments générateurs mâles et femelles du futur animal, . . ." In the developing winter egg the first germ-cells were found by Balbiani (1869-1872) at a later stage. They formed an oval structure which lay in the median line of the body and soon divided lengthwise into halves.

Witlaczil (1884), in viviparous Aphids, also recorded a single primitive germ-cell which separated from the inner surface of the blastoderm at the posterior end of the egg. This single cell grew rapidly, producing by division a group of round clear cells each containing a large nucleus. This mass of cells was attached to the tail-fold, and moved with this until it reached a position in the posterior dorsal region of the body. During the revolution of the embryo, the rudiment of the germ-glands divided, half going to either side of the abdomen.

The origin of the germ-cells of viviparous Aphids was investigated by Will (1888). This author found that a thickening took place in the lateral wall of the blastoderm. "Bald aber sieht man die obersten Zellen der verdickten Cylinderseite sich in besonderer Weise differenziren; sie wachsen sehr schnell zu ansehnlicher Grösse heran, nehmen polyedrische Gestalt an und zeichnen sich durch eine geringere Tinctionsfähigkeit ihres Plasmaleibes sowie eine andre Beschaffenheit des Kernes aus. Durch rege Theilung vermehren sie sich sehr lebhaft und stellen bald einen rundlichen Zellenhaufen, die Geschlechtsanlage, dar, welche in dieser Gestalt während der nächsten Entwicklungsstadien verharrt.

"Die Geschlechtszellen liegen auf der Grenze zwischen dem Entoderm, dem Ectoderm der Bauchplatte und dem gleich sich bildenden Mesoderm. Ueber ihre Zugehörigkeit zu einem der drei Keimblätter lässt sich streiten; mir genügt es vollkommen, zu constatiren, dass wir in ihnen indifferente Gebilde vor uns haben, die gerade dort entstehen, wo die drei Keimblätter an einander stossen."

### 4. *Hymenoptera.*

The Hymenoptera are represented in the literature of insect embryology by numerous papers chiefly on the bee. With the exception of the male of *Apis* (Petrunkewitsch, 1901) the germ-cells have not been traced back to as early a stage as in several other orders of insects.

Ganin (1869) was the first to investigate the germ-glands of the Hymenoptera. In *Platygaster* the rudiments of the germ-glands were described at the end of the first larval instar as separate thickenings on either side of the posterior end of the germ-band near the hind-intestine; between them arose a temporary unpaired elevation, "Geschlechtshügel," which disappeared during the second larval instar. In another Hymenopteron *Polynema,* Ganin found that the germ-glands developed from the undifferentiated cell mass of the tail-fold, and lay in the cavities of the last abdominal segments, where they remained during the larval period.

Bütschli (1870), in his work on the bee, described in a well developed embryo "nicht weit von den Rückenrändern der Leibeswandung jederseits eine durch ungefähr 5 Segmente sich erstreckende längliche Zellenmasse aus dicht gedrängten rundlichen mit grossen Kernen ausgestatteten Zellen bestehend," which he considered the rudiments of the germ-glands. Bütschli distinguished two layers in the germ-band of the bee, from the inner one of which, the entomesoderm, the primitive germ-cells were supposed to be derived.

Uljanin (1872), according to Brandt (1878), found in the larva of the bee, two kidney-shaped bodies lying on either side of the dorsal vessel; he considered these to be the female sexual organs.

In several species of Hymenoptera Dohrn (1876) "fand in jungen Larven die Anlagen der Ovarien als einen breiten birnförmigen Körper, dessen breite Fläche in acht fingerförmige Fortsätze ausgezogen war, deren vier oben und vier darunter liegen."

Ayers (1883) studied *Teleas,* a parasite in the egg of *Oecanthus niveus.* Here the germ-cells were budded off from the dorsal side of a posterior enlargement of the nerve cord which curved upward around the end of the mesenteron. A varying number of cells (two to six) were thus produced embedded in homogeneous protoplasm. "They appear in sections of hardened specimens as though formed endogenously within the substance of the still persisting mother cells. . . These are shortly separated from the nervous cord, but are connected to the blind end of the mesenteron by protoplasmic filaments, usually one to each mother cell."

The embryonic germ-glands of the bee were described by Grassi (1884) as two solid strands, extending from the fourth to the eighth abdominal segment. "Sono formazione mesodermica; nascono press' a poco ai confini tra il foglietto superficiale e il foglietto profondo del mesoderma. . . ."

The rudiments of the germ-glands were found by Carriere (1890) in *Chalicodoma muraria* at about the time when the first pair of Malpighian tubules appeared. They lay near the dorsal wall of the "Urhöhle" in the fifth and sixth abdominal segments.

Bugnion (1891) studied the postembryonic development of *Encyrtus fuscicollis.* The rudiments of the reproductive organs were found in the middle of larval life; they were oval structures lying on either side of the hind-intestine. During the second half of larval life the sexes could be distinguished; the testis remained small and round, while the ovaries became oval and larger.

The "Anlage" of the germ-glands were found by Kulagin (1897) in the parasitic Hymenopteron, *Platygaster herrickii,* lying near the hind-intestine of an embryo in which the mesodermal somites were forming. This rudiment was a paired structure composed of cells similar to those of the mesentoderm, the only difference being their

tendency to stain more intensely. "Dafür spricht erstens die frühe Absonderung der Geschlechtszellen, schon zu einer Zeit, wo das Meso- und Entoderm nicht scharf getrennt ist, und ferner die sehr grosse Aehnlichkeit bei den Zellelementen in den ersten Stadien ihrer Entstehung."

According to Carriere and Bürger (1897) the primitive germ-cells of *Chalicodoma muraria* were probably derived from cells of the mesodermal layer shortly after its appearance. A few cells in the dorsal wall of the somites of the third, fourth and fifth abdominal segments on either side of the body proliferated to form egg-shaped bodies. In further growth these germ-cells decreased in size as a result of multiplication. Later, they wandered from the third and fourth segments into the fifth, where they lay behind one another in their original succession.

In an endeavor to test the "Dzierzon theory," that the eggs which produce drone bees are normally unfertilized, Petrunkewitsch (1901-03) discovered some unusual maturation divisions. In "drone eggs" the first polar body passed through an equatorial division, each of its daughter nuclei containing one-half of the somatic number of chromosomes. The inner one of these daughter nuclei fused with the second polar body, which also contained one-half of the somatic number of chromosomes; the resultant nucleus with sixteen chromosomes, the "Richtungscopulationskern" passed through three divisions giving rise to eight "doppelkernige Zellen." After the blastoderm was completed, the products of these eight cells were found in the middle line near the dorsal surface of the egg, where the formation of the amnion had already begun; the nuclei of these cells were small, and lay embedded in dark staining cytoplasm. Later they were found just beneath the dorsal surface near the point of union of the amnion with the head-fold of the embryonic rudiment. They were next discovered between the epithelium of the mid-intestine and the ectoderm; from here they migrated into the cœlomic cavities, and finally, at the time of hatching, formed a "wellenartigen" strand, the germ-gland, extending through the third, fourth, fifth and sixth abdominal segments. The fertilized eggs of the bee were also examined by Petrunkewitsch, but no "Richtungscopulationskern" was present. In these eggs "entstehen die Genitaldrüsen aus Mesodermzellen, die in die Mesodermröhren von der Bauchseite hereindringen."

### 5. *Orthoptera.*

Many papers have appeared contributing to our knowledge of the germ-cells of the Orthoptera. Unfortunately, a large number of them are based on a study of one species, *Blatta germanica,* which is not favorable for this particular phase of embryological research.

We owe the first account of the primitive germ-cells of the Orthoptera to Ayers (1883). He found the rudiments of the germ-glands in *Oecanthus niveus* at a late embryonic stage after revolution of the embryo had taken place. "They are first seen as two irregular groups of amœboid cells, belonging to the splanchnic layer of the mesoderm on either side of the dorsal vessel." These groups of cells were transformed into ovaries in their primitive position.

Nusbaum (1886) considered the reproductive organs of *Periplaneta orientalis* of mesodermal origin.

Heymons (1890-91) made a detailed study of the origin and development of the germ-glands of *Blatta germanica.* He found in this insect a segmental origin of the germ-cells. They were identified as large cells, in the second to the seventh abdominal segments, which arose from the splanchnic layer of the mesoderm; new genital cells were continually added. Later a migration of the germ-cells took place into the cœlomic cavities, and afterward between the cells of the dorsal walls of the cœlomic sacs. Here a continuous strand of germ-cells was formed on either side of the body; these were situated in the dorsal wall of the primitive somites extending from the second into the eighth abdominal segment. Undifferentiated mesoderm cells were added to these strands from the dorsal wall of the cœlomic sacs, to form the epithelium of the germ-glands. The strands now shortened, and, by the lateral pushing of the germ-band around the yolk, were carried to the dorsal side of the egg where they continued their growth.

Heymons (1890) claimed to have discovered an hermaphroditic condition in the male genital organs of *Blatta* (*Phyllodromia*). "Es geht hieraus unzweifelhaft hervor, dass jener Theil der Genitalanlage beim Männchen, welcher nicht mit zur Bildung der Hodenfollikel verbraucht wird, die Anlage zu einer weiblichen Genitaldrüse darstellt." Other workers have not sustained Heymon's interpretation.

Several papers on the embryology of *Blatta* (*Phyllodromia*) *germanica* were published by Cholodkovsky (1890-91). This author differed in some points from Heymons (1890-91). He found the primitive germ-cells always lying in the dorsal wall of the cœlomic cavities pre-eminently if not exclusively in the fifth and sixth abdominal segments. The number of germ-cells increased either by division or by the addition of new cells which penetrated into the cœlom. As the embryo grew dorsalward around the yolk, the germ-glands were carried to a point on either side of and dorsal to the mid-intestine. Cholodkovsky did not agree with Heymons that the germ-cells differentiated from mesoderm-cells, but held that they probably were derived from yolk-cells.

*Stenobothrus variabilis* was studied by Graber (1891). The first distinct rudiments of the germ-glands were found as two large faintly stained cells differentiated from the visceral layer of the dorsal "Mesoblastdivertikel." At the end of the embryonic period the sexual organs appeared as two strands lying close to one another on the dorsal side of the posterior region of the mid-intestine.

The germ-glands of *Mantis religiosa* seemed to Graber delayed in their development as compared with those of *Stenobothrus*, for, in the former, they still retained their lateral position at the end of embryonic life.

Wheeler's (1893) paper on *Xiphidium ensiferum* and other Orthoptera contains a short account of the development of the germ-glands. In the above named species the first sign of the primitive germ-cells was not discovered until the somites were established as distinct sacs. The germ-cells at this time lay in the splanchnic walls of the somites of the first to the sixth abdominal segments; one group was found in the tenth abdominal segment. They were larger and paler than the mesoderm-cells and were thought to have been derived from the latter. A cluster of cells was formed by the mitotic division of these primary germ-cells. The somites that bore germ-cells each sent out a solid diverticulum which connected with the antecedent somite, thus producing a continuous strand. The hexametameric arrangement of the germ-cells disappeared as this strand shortened to form the ovary or testis. Wheeler noted the presence of germ-cells in the first abdominal segment of *Blatta germanica* in opposition to Heymon's statement that "Im ersten Abdominalsegment treten niemals Genitalzellen auf."

Heymons' earlier papers were supplemented by a re-examination (1895) of *Blatta* (*Phyllodromia*) *germanica* and a clear account of the development of other Orthoptera, *Periplaneta orientalis, Gryllus campestris, Gryllus domestica* and *Gryllotalpa vulgaris.*

In *Blatta* he was able to trace the germ-cells back to an earlier stage than recorded in his former paper (1891). When the posterior amniotic fold arose, a groove, "Geschlechtsgrube," appeared in the posterior end of the germ-band. Many cells became detached from the bottom of this groove and wandered into the interior; they moved singly and "sich teils zwischen den Mesodermzellen, teils über sie hinweg nach vorn bewegen." They assumed the character of the germ-cells only when they arrived at the visceral walls of the somites where they behaved just as previously stated (Heymons, 1891).

In *Periplaneta* a "Geschlechtsgrube" also appeared similar to that found in *Blatta;* cells separated from it and migrated by amœboid movements toward the anterior abdominal segments where they arranged themselves intersegmentally forming wedge-shaped accumulations between the cœlomic sacs. Contrary to the condition in *Blatta,* these could be distinguished as germ-cells shortly after they became detached from the ectoderm, Later these germ-cells separated to form two strands lying on either side of the body in the visceral walls of the second to the seventh abdominal segments; they acquired an epithelial layer derived from mesoderm-cells.

A "Geschlechtsgrube" was found by Heymons (1895) in both *Gryllus campestris* and *G. domesticus*, and the germ-cells arose in a manner similar to that in the Orthoptera previously described. These two species differed only in the fact that the germ-cells differentiated earlier in the former than in the latter. A long oval structure was produced by the accumulation of the primitive germ-cells; this body divided during the segmentation of the germ-band, one half going to either side, where it lay in the dorsal region of the second and third abdominal segments. Here the germ-gland was enclosed in an epithelium of mesoderm-cells.

*Gryllotalpa vulgaris* was not so thoroughly studied as the other species. Heymons, in this Orthopteron, found "nur sehr flache Einstülpung. Von dem Boden der Letzteren lösen sich Zellen los und wandern ein. Ich bin geneigt, diese Zellen als Geschlechtszellen anzusehen, obwohl sie das Aussehen von Mesodermzellen haben und ich ihr weiteres Schicksal nicht verfolgt habe."

## 6. *Coleoptera.*

The Coleoptera are represented in the literature on the embryology of insects by only a few papers; of these eight contain fragmentary accounts of the origin and early development of the germ-glands.

Heider (1889) found that in *Hydrophilus piceus* the germ-glands originated from the inner wall of the primitive abdominal segments on either side of the body. They arose as solid outgrowths from that part of the wall of the somites which lay between the place of origin of the Fettkörperband" and the splanchnic layer of the mesoderm.

The germ-glands of *Leptinotarsa* (*Doryphora*) *decemlineata* were described by Wheeler (1889) as follows: "These organs originate as two elongated thickenings of splanchnic mesoderm, one on each side projecting into the body cavity. Later they become rounded and are attached by a thin band of splanchnic mesoderm only." Wheeler found several cells in the posterior amniotic cavity of an embryo of *Leptinotarsa* which he concluded might be comparable to the pole-cells of Diptera. The origin and fate of these cells was not determined.

In *Melolontha vulgaris,* the sexual organs were found by Voeltzkow, (1889) "zu der Zeit, wo die Darmwülste sich am Bauch zu schliessen beginnen und die Leibeshöhle sich fertig gebildet hat. Sie werden vom Mesoderm aus gebildet und liegen am hinteren Ende des Eies als ein Paar birnförmige Gebilde, umgeben von einer starken, ringförmigen Zellenmasse mit grossen Kernen. . . Später rücken sie etwas nach dem Rücken hinauf und liegen rechts und links vom Rückengefäss."

Three species of beetles, *Hydrophilus piceus, Melolontha vulgaris* and *Lina tremulæ* received brief mention by Graber (1891). In *Hydrophilus* the two germ-glands were found lying near each other on the dorsal wall of the intestine, close to the proctodeum. In *Melolontha* "Die Gonaden erscheinen hier sehr frühzeitig als mit dem Visceralblatt verbundene gestielte Körper, an denen man wieder ein Zellepithel und mehrere grössere und kleinere Inhaltszellen unterscheiden kann." In *Lina* the reproductive organs were discovered anterior to the proctodeum in connection with the "Darmfaserblatt." They appeared very similar to the corresponding organs described by Wheeler (1889) in *Leptinotarsa.*

In a Russian paper, Nusbaum (1891) has figured at the posterior pole of the egg of the oil beetle, *Melœ proscarabœus,* a wedge-shaped structure designated as an "accumulatio plasmatis et nucleorum in posteriore polo ovi." This nucleated mass occupied the position of, and is very similar in appearance to, the group of pole-cells and pseudoblastodermic nuclei shown in Fig. 25; in this species there probably occurred an early development of the germ-cells, such as has been found in Chrysomelid beetles.

The embryological development of the following species of Chrysomelidæ was studied by Lecaillon (1898); *Clytra læviuscula, Gastrophysa raphani, Chrysomela menthastri, Lina populi, L. tremulæ Agelastica alni.* In *Clytra,* the principal form examined, Lecaillon found the first nuclei which arrived at the posterior pole of the egg to become the primitive germ-cells; these could be distinguished from neighboring cells by their large size, larger nuclei, and darker cytoplasm. The germ-cell nuclei did not stop when they reached the surface of the egg, but passed outside and became separated from it; their number increased "peu à peu par suite de l'arrivée de nouvelles cellules périphériques et aussi sans doute de la division des premières cellules détachées du pôle de l'œuf." The germ-cells then started to re-enter the egg, retarding, by this migration, the formation of the blastoderm at this point. "Finalement, le blastoderme achève de se former au pôle posterieur de l'œuf, et alors les cellules sexuelles se trouvent groupées . . . entre le vitellus et l'enveloppe blastodermique." At the end of segmentation, the germ-cells were found pressed against the inner surface of the germ-band, just in front of the posterior end of the egg; here they remained during the formation of the mesoderm. After the mesodermal somites were completed, the germ-cells penetrated into them, and formed two cylindrical groups, the germ-glands. These were then carried by the lateral growth of the embryo to a point near the median dorsal line.

The above described processes also took place in *Chrysomela menthastri, Lina populi* and *L. tremulæ.* The primitive germ-cells of *Gastrophysa* arose as in *Clytra,* but many of them remained outside of the egg and were later found in the posterior amniotic cavity, "dans le sillon profond qui se trouve sur le milieu de sa paroi interne. Avant la fermeture du sillon, les cellules y pénétrant et ses trouvent ensuite en dedans de la couche ectodermique." No observations were made by Lecaillon upon the precocious appearance of the germ-cells in *Agelastica alni.*

Several species of Chrysomelid beetles were studied by Friederichs (1906). This author discovered that the cleavage nuclei in *Donacia crassipes* reached the posterior later than the anterior end of the egg; the reverse is the rule in species of allied genera. After the blastoderm was formed "an der Ventralseite unmittelbar seitlich vor dem Pol, findet eine besonders lebhafte Zellvermehrung statt, so dass einzelne Zellen aus dem Blastodermverband heraus und ins Innere gedrängt werden." These, the primitive germ-cells, were not very different in *Donacia* from blastoderm-cells, but in *Timarcha nicæensis* and *Chrysomela marginata* they were distinguished by the larger size and darker color of their nuclei. The blastoderm (Ectoderm) became interrupted at the point of origin of the germ-cells, an invagination being found similar to the "Geschlechtsgrube" of Orthoptera (Heymons, 1895). The germ-cells remained just inside of this groove; by the lengthening of the embryonic rudiment they were carried to a point near the mid-dorsal region of the egg. Here they were found at the end of the tail-fold, lying between the ectoderm and the yolk. The germ-cells, as well as the other cells of the embryo, gave off “Paracytoide” which helped dissolve the yolk. These “Paracytoide” arose either from degenerated nuclei or from “Kernzerlegung” and contained “keine Chromosomen, sondern nur Trophachromatin (Chromidialkerne)”.

The most recent contribution to the development of the germglands of the Coleoptera is that of Saling (1907). This paper deals exclusively with the origin and growth of the reproductive organs of *Tenebrio molitor.* Saling did not find in this species such a precocious differentiation of germ-cells as Lecaillon (1898) recorded for Chrysomelid beetles, but says: “Dagegen halte ich für wahrscheinlich, dass ihre Loslösung vom Ectoderm am hinteren Keimstreifende erfolgt, sobald sich die hintere Amnionfalte erhebt und die Mesodermbildung im Gange ist. Beim Vorwärtsdringen der sich segmental anordnenden Mesodermmasse schiebt sich auch die noch unpaare Genitalanlage weiter nach vorn und gelangt vor Ausbildung der Ursegmente an die Grenze des sechsten und siebenten Abdominalsegments. Durch eine Teilung in lateraler Richtung wird sie paarig und tritt mit den inzwischen ausgebildeten Cölomsäcken des siebenten Abdominalsegments in Verbindung. Erst von diesem Zeitpunkt an ist die Genitalanlage bei *Tenebrio molitor* mit Sicherheit zu erkennen.” The germ-cells penetrated through the median wall of the abdominal segments to the cœlomic cavities gathering a mesodermal epithelium during this migration. Sex-differentiation took place shortly before the end of the embryonic period.

## 7. *Neuroptera.*

The origin of the germ-glands in the Odonata and Ephemerida is still unknown. Heymons (1896) was unable to find the germglands in the embryos of the dragon-fly, but in the larva discovered them lying on either side of the intestine. They were spindle-shaped and composed of only a few cells. It was also impossible to find the germ-glands of *Ephermera* during embryonic life. Heymons concluded “dass bei den Odonaten und Ephemeriden die Differenzirung der Geschlechtsdrüsen nicht beim Embryo, sondern erst bei der Larve sich abspielt.”

## 8. *Dermaptera.*

The primitive germ-cells were identified by Heymons (1895) in *Forficula auricularia* soon after the blastoderm was completed, forming a group at the posterior pole of the egg. At this time they could not be distinguished from the blastoderm-cells, but soon their nuclei became larger and clearer. The germ-cells increased rapidly by division and formed a spherical body, the genital rudiment. "Paracyten" were abundant near the germ-cells, and "Auch einzelne Genitalzellen pflegen nicht selten zu degeneriren, und zwar unter denselben Erscheinungen, die wir an den Paracyten kennen gelernt haben." The genital rudiment was pushed anteriorly near the dorsal surface of the body, by the lengthening of the germ-band. When the primitive segments appeared, the germ-cells, which lay in the tenth to the eleventh segments, were, by the bending of the posterior end of the body, forced into the ninth segment. They now separated from one another and migrated anteriorly by means of amœboid movements until they reached the sixth and seventh abdominal segments. Sometimes a few germ-cells were left behind in segments eight, nine, ten, or eleven. Now the germ-cells separated, half going to either side of the embryo, and moved anteriorly into the third to the seventh abdominal segments. Most of the germ-cells of the male remained in segments five, six and seven; those of the female were distributed approximately uniformly through segments three to seven.

## 9. *Aptera.*

Heymons (1897) has given a clear account of the primitive germ-cells in a *Thysanuran, Lepisma saccharina.* In this insect a knob-like projection was observed at the hinder end of the germ-band; this projection was composed of cells with large nuclei containing less chromatin than the nuclei of the mesoderm-cells, which had also begun to appear. These larger nuclei were interpreted as germ-cells and arose from the ectoderm. The primitive germ-cells migrated just as they were found to do in Orthoptera (Heymons, 1895), "Stets gelangen die Geschlechtszellen an die dorsalen Ursegmentwandungen, dringen in dieselben ein und bilden zusammen mit den

Mesodermzellen der letzteren die Genitalfollikel." A strand of cells was formed on either side of the body and from each, in the female, five pairs of follicles arose and were distributed in segmental order from the second to the sixth abdominal segment. In the male, double pairs of follicles occurred in the fourth to the sixth abdominal segments. There were thus produced twelve "Hodenfollikel" and ten egg-tubes.

Very little attention has been directed to the early development of the germ-glands in the Collembola, but several embryologists have, however, made observations on the later stages of these organs.

Claypole (1898) described two methods of origin of the germ-cells in *Anurida maritima.* In the first case a germ-cell was found lying in the cavity of the mesoblastic somite of the second abdominal segment; this gave rise to a mass of cells, the germ-gland, situated between the splanchnic and somatic layers of the mesoderm. Later, the germ-gland appeared to break through the mesoderm and come in contact with the yolk. The ovaries which developed in this way were found to contain many yolk globules lying among the germ-cells.

In the second case, Claypole states that a single germ-cell passed out from the wall of the mesoblastic somite into the yolk; it produced an irregular mass of cells lying partly in the mesoderm and partly in the yolk. The cells of this group migrated inward becoming scattered among the yolk-globules. The next period in the history of these cells was not found. The final stage found by this author represented the germ-gland of the male; it was an oblong structure directly connected with a large irregular sac of yolk.

The ovaries of ten species of Collembola were studied by Lecaillon (1901). In *Sira nigromaculata,* just after hatching, the female sex-organs were described as "deux petites masses ovoides placées dans la cavité générale du corps, à peu près a égale distance de l'extrémité antérieure et de l'extrémité postérieure de l'abdomen." They were situated ventrally between the third and fourth abdominal segments. The germ-glands of *Tomocerus plumbeus* and *Templetonia nitida* were found to occupy a position similar to that described above. In *Templetonia,* however, "les dimensions de la chambre gonadiale surpassent notablement celles du même organe chez les deux espèces précédentes."

## III. OBSERVATIONS.

### 1. *The Pole Disc.*

A disc-shaped mass of dark staining granules is present at the posterior end of the freshly laid eggs of both *Calligrapha* and *Leptinotarsa;* these granules lie suspended in the inner stratum of the "Keimhautblastem." This layer of granules, which I shall call the pole-disc, plays one of the most important rôles in the genesis of the pole-cells; on this account I shall in this place describe its early characteristics, leaving its later history to the succeeding chapter.

Unfortunately, material was not at hand with which to trace the origin of this pole-disc, the earliest stage in my preparation (Fig. 2) being the eggs of *Leptinotarsa* taken from the oviduct just before deposition. At this time the pole-disc is present in the position it occupies throughout the entire course of the early cleavage of the egg. Under low magnification it appears in longitudinal sections as a dark irregular line lying just within the surface at the posterior end of the egg; under high powers, however, it is seen to be granular in structure. In the section figured (Fig. 2) it occupies the innermost portion of the "Keimhautblastem;" its granules are grouped together in small masses giving the entire pole-disc the appearance of a broken strand. These granules are easily distinguished from the cytoplasm in which they are suspended, by their large size and susceptibility to various stains; they appear to be arranged around small vacuoles which vary in size in the different preparations examined, irrespective of the age of the egg. Thus the granules in the pole-disc under consideration (Fig. 2) are crowded as closely together as they ever become, while in other eggs taken from the same batch they were widely separated.

Fig. 1 represents the posterior end of an egg of *Calligrapha;* here we see that the pole-disc occupies about one-eighth of the total area. The central part of the disc is denser than it is in any other region of it except the periphery, where an irregular margin is produced by numerous dark projections. Longitudinal sections of the pole-disc (Fig. 3) explain this difference in density, as it is found to be thickest in the center, and to be thrown into irregular folds at the ends causing these portions to appear darker when seen in surface view.

The condition exhibited by most of the discs examined is represented in Fig. 3; the granules are not crowded closely together as in the disc described above (Fig. 2), but lie distinct from one another, forming an alveolar-like structure (a network in longitudinal section). Large vacuoles occur near the outer and inner surfaces of the disc, and in some cases yolk-globules come almost in contact with its upper side. Scattered about in the cytoplasm near the margin of the disc, are a number of its granules which have become separated from the main structure.

A third disc (Fig. 4) illustrates another condition which is not unusual, especially in the later cleavage stages of the egg. The granules have apparently lost their alveolar arrangement, now being diffused throughout a greater portion of the "Keimhautblastem." Within this disc are several large vacuoles surrounded by regular layers of granules.

The granules of the pole-disc are very susceptible to stains; in hæmatoxylin, thionin and gentian violet a color was obtained as deep as that of the chromatin in either dividing nuclei or those in a resting condition; they stained in orange G more intensely than the surrounding cytoplasm and almost as deeply as the yolk-globules. Other stains failed to bring out any further variations in the results.

### 2. *The Genesis of the Pole-Cells.*

Before describing the genesis of the pole-cells of *Calligrapha multipunctata* it seems desirable to give a brief account of the maturation, fertilization and early cleavage of the egg.

Eggs that have just been laid contain polar bodies in various phases of formation; these are given off into a thickening of the "Keimhautblastem" at a point slightly anterior to the median transverse axis of the egg. Here they remain and later disintegrate.

The female pronucleus lies in an amœboid accumulation of cytoplasm among the yolk-globules. It moves inward and conjugates with the male pronucleus at a point level with the polar bodies.

Here the first cleavage divisions take place. A number of the earliest cleavage stages were found (2, 4, 6, 16, etc.) in all of which the majority of the nuclei were nearer the anterior than the posterior pole. This anterior position of the early cleavage nuclei has already been described for Coleoptera in *Hydrophilus* (Heider, 1889), and in many insects belonging to other orders.

As cleavage progresses a separation of the nuclei into two sections takes place. The nuclei of one group form a more or less regular layer equidistant from the periphery at all points except the posterior end; here a space wider than elsewhere separates them from the surface of the egg. This layer is composed of preblastodermic nuclei (by nucleus is meant the nucleus plus its accompanying cytoplasm) which move outward, fuse with the "Keimhautblastem" and produce the blastoderm. The nuclei of the other group (vitellophags) remain behind scattered throughout the yolk.

When the preblastodermic nuclei have almost reached the peripheral layer of cytoplasm (Fig. 5) it is possible to distinguish those whose descendants will come in contact with the pole-disc from the neighboring nuclei which will produce ordinary blastoderm-cells. This distinction can be made more easily in a polar surface view (Fig. 12) which shows the entire pole-disc. Such a view discloses eight nuclei lying directly under the central area of dark granules. These nuclei, as we shall show later, divide twice before reaching the periphery of the egg. Some of the nuclei thus produced will, however, pass outside the margin of the disc and take part in blastoderm formation; the others, which will enter this granular area and become the pole-cells, can be traced back to the row of four nuclei which lie in the center of the pole-disc nearest the surface of the egg. At this stage (Fig. 5) all of the nuclei within the egg are similar, the various stains used (see the chapter on methods) failing to bring out any differences in structure. Differentiation takes place only when those nuclei in the posterior region fuse with the "Keimhautblastem." Continued division brings the preblastoderm-nuclei into the position shown in Fig. 6, where they again increase in number by mitosis. In the section figured (Fig. 6) all of the nuclei are in the prophases of mitosis, and each of those nearest the pole-disc (Fig. 6, *a*) has its chromatin partially arranged in an equatorial plate. An enlarged view of the two nuclei which lie nearest the posterior pole (Fig. 18) shows that each is surrounded by an amœboid mass of cytoplasm with many fine pseudopodia-like processes extending outward in all directions, finally becoming lost among the yolk-globules. The nuclear membrane of each nucleus has disappeared, but its former position is clearly marked by the difference in density between the cytoplasm and the nuclear sap; a fusion of these two substances has not yet taken place. No nucleolus is visible.

After this division is completed, the daughter nuclei are closer together and nearer the surface of the egg (Fig. 7, *a*). The two nuclei with their accompanying cytoplasm, which now lie closest to the posterior pole (Fig. 19), have finally come in contact by means of their pseudopodia, with the "Keimhautblastem." These two nuclei are in a resting condition; their chromatin is evenly distributed throughout, the chromomeres lying in a linin reticulum singly or in groups of from two to six or more. One small nucleolus is present. All the other nuclei in the egg are similar in appearance at this time, and no differences in size or structure could be distinguished. It is not unusual to find all of the nuclei within the egg in repose at the same time, as mitosis is very rapid, and the subsequent resting period relatively long; thus, although nuclei in different regions are often found to be in different phases of division, nearly all of the sections made from eggs in preblastodermic stages show every nucleus in a resting condition. Each nucleus is in repose at this time (Fig. 19), but the cytoplasm accompanying it is still actively engaged in its migration toward the periphery. The cytoplasm surrounding the nuclei reaches the "Keimhautblastem" and the granular pole-disc simultaneously, the latter at this point occupying the inner portion of the former as already described in the preceding chapter.

Each nucleated mass of cytoplasm that comes within the limits of the pole-disc presses outward a mass of granules equal to the area of its fore-end. The whole disc is thus indented in as many places as there are protoplasmic masses striking it (Fig. 8 and Stage A). Fig. 20 illustrates two stages (*a* and *b*) in the early genesis of the pole-cells, and also shows at the extreme right a third nucleus (c) which lies outside of the pole-disc and is destined to become a part of the blastoderm. The central nucleus of this trio (*b*) has pushed a part of the pole-disc outward and forced the granules into a cap (a half-moon in section) which extends half-way up its sides. The granules are now not as widely separated from one another as we found them in a previous stage (Fig. 3); they have become more densely packed due to the pressure exerted by the migrating pole-cell nucleus in its effort to break through the "Keimhautblastem" (compare Figs. 4, 18 and 19). The neighboring pole-cell nucleus (Fig. 20, *a*) is in a somewhat more advanced stage; it shows that the granules covering its outer surface become, in part, pushed away from this region and gradually extend around its sides until they cover all of the surface except the innermost portion. All of the granules of the pole-disc do not adhere to the pole-cells, some being left behind within the remains of the "Keimhautblastem."

A surface view of the posterior end of the egg at this stage shows plainly eight pairs of pole-cell nuclei (Fig. 13); an enclosing layer of the dark staining granules is suspended within each of these enabling one to distinguish easily the pole-cell nuclei from the adjacent blastoderm-nuclei. This paired condition of the pole-cell nuclei is accounted for by the fact that since the division of the eight nuclei of the previous generation, the daughter nuclei have not yet separated. In one case where the daughter nuclei have drawn some distance apart from each other (Fig. 13, *b*) a wide strand of cytoplasm is seen connecting the two. A few of the neighboring blastoderm nuclei, which have passed near the edge of the pole-disc, have also carried with them some of the granules which are seen embedded in their adjacent sides (Fig. 13, *a*). The nuclei of the blastoderm are slower than the pole-cell nuclei in reaching the surface, and have hardly begun to protrude when the latter have almost, or, in some cases, entirely separated from the egg (Fig. 9). Each pole-cell nucleus is now completely surrounded by a layer of granules more dense at the side than at either end (Fig. 21). After reaching the position last described (Fig. 9) all of the pole-cell nuclei, except in a few cases where one of them has been delayed (Figs. 26 and 22) become entirely separated from the egg, and lie in a single layer between the remains of the "Keimhautblastem" and the vitelline membrane. They now vary from one another both in the size of the entire cell and in that of the nucleus. The long cytoplasmic processes which were characteristic of the pole-cell nuclei while within the egg have disappeared; they have undoubtedly been retracted until they now form a few blunt projections still suggestive of pseudopodia (Fig. 22, *b, c*). The peripheral portion of each cell consists of a lightly staining vacuolated layer, which was formerly, before the pole-cell nuclei separated from the egg, the outermost stratum of the "Keimhautblastem" (Fig. 20, *vac. st.*). This layer has spread completely over the surface of the protruded pole-cells, and in consequence of this increase in area covered, has become thinner; its structure, however, is still the same as when it constituted a part of the egg (Fig. 3, *vac. st.*). This is doubtless due to the granular layer which, by its intervention, impedes the fusion of the vacuolated portion with the denser cytoplasm within the cell.

Just within the outer stratum is the granular layer; this no longer appears evenly distributed (Fig. 21), but now seems to be massed in certain places and absent elsewhere, as shown in Fig. 23, *pdg.* A close examination of sections (Fig. 22, *pdg*) shows, however, that the granules completely surround the nucleus as before but have accumulated in the regions of the pseudopodia. These accumulations are often found in the granular layer of other stages and may even be present in the pole-disc before the pole-cells are protruded (see also Ritter, 1890, Taf. XVI, Fig. 3, and Noack, 1901, Taf. II, Fig. 19).

Between the granular layer and the nucleus is a homogeneous stratum of cytoplasm; this consists of all the protoplasm that surrounded the nucleus before it reached the periphery plus that layer of the "Keimhautblastem" in which the granules were suspended. The nuclei of one of these cells (Fig. 22, *b*) shows a spireme, the other (Fig. 22, *c*) a later stage in which the spireme has already segmented into a number of chromosomes.

As stated above, it sometimes happens that the pole-cell nuclei do not all reach the "Keimhautblastem" simultaneously; those which are delayed have difficulty in collecting enough cytoplasm for their needs since most of the peripheral layer has already been carried away by the nuclei that first reach the surface. One of these delayed nuclei (Fig. 22, *a*) shows, embedded in its accompanying cytoplasm, a number of small yolk-globules which have not yet been dissolved.

I have already said that eight pairs of pole-cells protrude from the posterior end of the egg and from a single layer there (Fig. 13); these I shall speak of as the primary pole-cells. It has also been shown that when these sixteen primary pole-cells have become completely separated from the egg they are in the prophases of mitosis (Fig. 22). These now undergo their first division, giving rise to the secondary pole-cells which immediately divide again. However, before this second division is completed an accurate count of the number of pole-cells is possible as the presence of granules easily distinguishes them from those of the blastoderm. The number of pole-cells ranges from thirty-two to forty at this stage, thirty-four being present in the specimen illustrated (Fig. 14); only one of these is dividing (Fig. 17) showing that mitosis does not occur in all at the same time. A longitudinal section through an egg taken from the same batch as that just described also shows one pole-cell in a similar condition (Fig. 23, *a*). We conclude from this that the sixteen primary pole-cells have divided, resulting in thirty-two, and that two of these secondary pole-cells have produced daughter cells, thus bringing the total number to thirty-four. During the division of the pole-cells (Fig. 17) all of the granules separate into two approximately equal groups, which form a thin layer closely applied to the cell boundary at either end.

The pole-cells just previous to their second and final division, can, as already mentioned, be distinguished from the adjacent blastoderm-cells by the presence within the cytoplasm of the dark-staining granules of the pole-disc; they also at this stage show a difference in the structure of their nuclei. One cell of each kind, pole and blastoderm, is shown highly magnified in Fig. 28. Here we see that the nucleus of the pole-cell (p.c.) is the larger; it contains a relatively few rod-like pieces of chromatin which are most abundant near the nuclear membrane. In contrast to this, the smaller nucleus of the blastoderm-cell (bl.c.) is closely packed with chromomeres, and no nucleolus, such as is present in the pole-cell nucleus, is visible.

When the second mitosis is completed, it is difficult to determine the exact number of pole-cells, owing to their irregular arrangement. A superficial view of the posterior end of the egg discloses (Fig. 15) sixty-three pole-cells, one, the sixty-fourth, probably being hidden from sight by overlying cells. In lateral surface view (Fig. 16) the pole-cells are seen as a cap closely applied to the posterior end of the egg. Longitudinal sections (Figs. 23-25) demonstrate that they do not lie free in the polar cavity as described in *Chironomus* (Ritter, 1890, and others), but that those nearest the egg occupy an indentation in its end, in which position they are probably held by the vitelline membrane and the chorion (Balbiani, 1885, in *Chironomus*).

The entire surface of the egg, in the stage just described, is found to be covered by the blastoderm, except the area at the posterior end through which the primary pole-cells passed. Just within this area are seen (Fig. 25) a number of nuclei similar to those of the neighboring blastoderm-cells; they are not, as the latter, separated from one another by cell boundaries, but form an irregular mass, a syncytium. A group of nuclei similar in position and appearance has been noted by Ritter (1890) in *Chironomus* and by Noack (1901) in *Calliphora;* both authors maintain that these are yolk-nuclei. Lecaillon (1898) figures them in an egg of *Lina populi,* but does not mention them in the text (his Fig. 16). Voeltzkow (1889) found a few nuclei in eggs of *Musca* lying in the same region. He calls them inwandering blastoderm-cells. I hope to prove that they are the nuclei of blastoderm-cells, which, owing to the presence of the pole-cells, have been prevented from taking part in the formation of the blastoderm. In the stages figured (Figs. 6-11) no vitellophags are present near the posterior end of the egg from which this group could have arisen. Furthermore, no similar groups of nuclei are to be found at other places in the egg until a much later period of development; then, there may be found irregularly scattered throughout the yolk, small masses of cytoplasm, each containing three or four yolk-nuclei. A comparison of Figs. 23 and 24 shows that in the younger stages

(Fig. 23) the blastoderm ends abruptly at the place where it meets the pole-cells; in the older stage (Fig. 24) it has apparently pushed past the latter on all sides, and now projects obliquely upward into the yolk. A still later stage (Fig. 25) shows that these projecting ends of the blastoderm finally meet, thus forming a structure which in longitudinal section appears as an inverted V, but in reality is a cone-shaped plug extending into the yolk. At first the nuclei are arranged near the surface of this cone in a fairly regular row; some of them, however, soon become displaced. By the time the genesis of the pole-cells is completed, these nuclei form an irregular group, just within the egg opposite the pole-cells with which they are connected by a mass of cytoplasm. We shall see later that they remain thus in communication with the pole-cells for a long period of embryonic growth. *In toto* preparations show this group lying within the yolk in the above described position (Fig. 16, *ps. bl. n.*).

### 3. *The History of the Pole-Cells Until the Sex of the Embryo Can be Recognized.*

In order to follow the history of the pole-cells, it is necessary to describe the development of the germ-band. The blastoderm, as stated in the last chapter, is present at the conclusion of the pole-cell formation, as a single layer of regularly arranged cells covering the entire surface of the egg, except a small area at the posterior end. The first change noticed in the blastoderm is a crowding together of the cells on the ventral surface of the egg. This results in the formation of a broad longitudinal band of closely aggregated cells, the ventral plate (Stage C). The edges of this plate are thrown up into two folds; these spread out in the posterior region extending to the end of the egg (Stage D), where they pass around the pole-cells and meet on the dorsal surface (Fig. 31). The entire ventral plate now decreases both in length and in breadth; during this contraction a longitudinal concavity, the ventral groove, appears (Stage E). By this shortening of the germ-band, the pole-cells are carried from their former position at the end of the egg (Stage B) to a point slightly anterior, on the ventral surface (Stage E); here they occupy a cup-shaped depression in the ventral groove. The ventral plate continues to decrease in length; this contraction combined with the above mentioned depression, produces a deep cavity at the posterior end of the groove in which the pole-cells lie (Fig. 29). A lateral view of the same egg (Fig. 30) shows part of the pole-cells concealed within this cavity.

The germ-band can now be recognized; it covers the entire ventral surface of the egg except a wedge-shaped area anterior to the groove (Fig. 29). A lateral view of an *in toto* preparation shows the cephalic region of the germ-band already clearly indicated as a large lateral lobe (Fig. 30). The ventral groove now becomes narrower except at its posterior end; here a comparatively large opening remains (Fig. 32, *a*; Stage F), which, since the last stage (Fig. 29), has moved some distance forward. The floor of the groove has at this point invaginated to produce a cavity which extends obliquely upward into the yolk (Stage G). At the bottom of this cavity we find the pole-cells. They now lie entirely below the surface of the egg, partly hidden under the closely opposed lateral folds (Fig. 32 lf.)

A sagittal section of an egg in this stage gives a good idea of the structure of the ventral plate (Fig. 33). Near the anterior end we can distinguish the beginning of an invagination (*a*) which will become the stomodæum. At the posterior end is noticed a much deeper depression which contains the pole-cells lying at the bottom near the entrance of a distinct canal, the "Polzellencanal" of Escherich (1900). This canal is that opening in the blastoderm at the posterior end of the egg, which was caused at an earlier period (see preceding chapter, Figs. 23 and 25) by the protrusion of the pole-cells. It can be determined from transverse sections that a posterior depression in the ventral groove is formed by the arching over of the lateral folds, thus producing a flask-shaped cavity (Fig. 34, *a*). The pole-cells lie near the pole-cell canal which contains a mass of cytoplasm connecting them with the pseudoblastodermic nuclei within the egg.

The next stage of development (Stage H) shows a still narrower germ-band already displaying signs of segmentation. The posterior amnioserosal fold has grown forward to cover almost half of the embryo, and is much further advanced than the anterior fold which has just begun to appear at the lateral edges of the cephalic lobes. The flask-shaped depression in the floor of the ventral groove, which was noticed in the preceding stage (Stage G) has increased in depth, forming the posterior amniotic cavity. The posterior end of the germ-band has forced its way through the yolk in this region, becoming a distinct tail-fold (Stage H).

Sufficient *Calligrapha* material was not at hand for a study of sections of this stage (Stage H), but eggs of *Leptinotarsa,* which pass through a similar course of development, were obtained in great abundance; Figs. 35 and 36 were made from eggs of the latter species. A sagittal section of the tail-fold (Fig. 36) shows five pole-cells situated at the end of the amniotic cavity; three other pole-cells have already passed through the pole-cell canal, while another has just commenced its journey. This is the first evidence we have found of the migration of the pole-cells from the outside into the embryo.

A transverse section of another egg (*Leptinotarsa,* Fig. 35) shows a similar migration of the pole-cells; in this preparation a number of pole-cells are lying at the bottom of what was formerly the ventral groove, a few having already entered the pole-cell canal. In both this and the preceding section, the pseudoblastodermic nuclei are fewer in number than in eggs of *Calligrapha.*

An embryo (Stage J) slightly older than that just described exhibits the following changes: first, the amnioserosal folds have almost completely overgrown the germ-band; second, segmentation has become more noticeable; and third, the tail-fold has penetrated still farther forward into the yolk. A sagittal section through the tail-fold (Fig. 37) reveals a well developed mesodermal layer just inside of the ectoderm. A number of pole-cells still remain in the posterior amniotic cavity, although more of them have passed through the pole-cell canal than in the embryo last figured (Stage H). The pseudoblastodermic nuclei which are still present show signs of disintegration.

Such great differences in structure are now apparent between the pole- and ectoderm-cells that one of each taken from Fig. 37 has been drawn much enlarged (Fig. 55). The pole-cell is nearly spherical; its homogeneous cytoplasm stains lightly; and its nucleus contains besides a nucleolus a number of small groups of chromomeres. The ectoderm-cell, on the other hand, is considerably larger; its cytoplasm stains more deeply and contains several vacuoles, and its nucleus, which is without a visible nucleolus, is completely filled with chromomeres. The relations of the parts of the tail-fold are at this stage most easily understood in transverse sections. In Fig. 38 we can still distinctly see the lateral folds (lf) of the ventral plate; these enclose the flask-shaped ventral groove (a) within which are the pole-cells migrating through the pole-cell canal. The amnion which arches over the structures is separated from the dorsal serosa by a thin layer of yolk. The pseudoblastodermic nuclei are still present within the egg.

The segmentation of the germ-band and the lengthening of the entire embryo now progresses rapidly (Stage K). The cephalic extremity extends completely over the anterior end of the egg, and may be seen covering part of the dorsal surface. The tail-fold has extended a little more than half way up on the dorsal surface of the egg. The segments of the head, thorax and abdomen are visible at this time. The pole-cells have changed their position very little, but a larger number of them are now found inside of the embryo scattered among the mesoderm-cells. A transverse section (Fig. 47) now shows a fairly regular row of pole-cells migrating through the pole-cell canal. The pseudoblastodermic nuclei which have decreased in number by disintegration are represented from this time on only by an occasional nucleus, finally disappearing altogether.

Our next stage (Stage L) is an important one in the history of the pole-cells. The embryo has become more deeply segmented and its appendages are now evident. The tail-fold which has begun to recede is shown in sagittal section in Fig. 40. In most cases all of the pole-cells have by this time migrated within the embryo, but in the section figured two of them are just entering the pole-cell canal which has become much shortened. A transverse section through the last abdominal segment (Fig. 48) reveals the fact that the pole-cells, as soon as they have entered the embryo, begin to separate into two groups; three of them, in the figure, are present at either side of the median line. A reconstruction made from several series of transverse, sagittal and frontal sections, shows the pole-cells distributed on either side of the last two segments of the tail-fold (Stage L).

The next embryo I shall describe (Stage M) has broadened throughout its entire length; it has also shortened, this being especially noticeable in the posterior portion. This contraction brings the tail-fold nearer the posterior end of the egg than we found it in the previous stage (Stage L). Now that the pole-cells have become an integral part of the embryo I shall call them germ-cells. These still lie in the last two abdominal segments, but have become partially surrounded by mesodermal-cells (Fig. 49). They appear in sagittal section (Fig. 41) to be closer to one another than we found them in Stage L (Fig. 40). In transverse section (Fig. 49) they are shown more clearly separated than before into two groups lying on either side of the tail-fold. Part of them have reached the inner end of the cœlomic cavity, while the others are apparently moving in that direction. Three kinds of cell (germ-, mesoderm- and ectoderm-cells) from Fig. 41 are shown much enlarged in Fig. 57. The germ-cells still exhibit all the characteristics that the pole-cells formerly possessed, and, in addition, contain a second nucleolus. The cells of the mesoderm are smaller; they appear darker than the germ-cells due to a greater susceptibility of their cytoplasm to stains, and the larger relative number of chromomeres in their nuclei. The columnar ectoderm-cells contain cytoplasm which is even darker than that of the mesoderm-cells, and their oval nuclei possess a smaller number of chromomeres, regularly distributed. The ease with which these different cells can be distinguished is evident from a glance at the illustration (Fig. 57).

A transverse section of an embryo slightly older than Stage M (Fig. 50) shows four germ-cells lying in a single row close to one another; they all have now reached a point near the inner margin of the cœlom and have acquired an epithelial covering of mesoderm-cells. A further shortening of the embryo brings the tail-fold close to the posterior end of the egg (Stage N). The germ-cells, still situated in the last two abdominal segments (Fig. 42), have crowded close together; they do not form, as in younger stages, a loose strand on either side of the body, but now constitute a distinct organ, the germ-gland.

After the contraction of the embryo is completed, the tail-fold no longer exists as such; what was formerly the end of it is now coincident with the end of the egg (Stage O). The posterior abdominal portion of this embryo is shown in sagittal section in Fig. 43. The germ-gland lies between the splanchnic and somatic layers of the mesoderm: its cells have moved closer together forming a more compact organ than before. A frontal section clearly shows its position relative to the other parts of the body (Fig. 54).

Figs. 51, 52 and 53 are from transverse sections of embryos seventy-five hours, eighty-six hours and one hundred five hours old respectively; these illustrate three stages in the path of the germ-glands as they are carried from the ventral to the dorsal side of the body by the lateral growth of the embryo around the yolk. A sagittal section of an eighty-six hour old embryo (Fig. 44) when compared with that of an earlier stage (Fig. 43) also demonstrates the same phenomena.

In the oldest stage figured (one hundred five hours, Fig. 45) it is possible to distinguish the sexes; the male gland is recognized by a constriction which, appearing in its middle region, gives to it a dumb-bell shape; the female organ is distinguished both in transverse (Fig. 53) and in sagittal (Fig. 46) sections by the presence of the developing terminal filaments (*tf.*).

## IV. General Considerations.

### 1. *The Granules of the Pole-Disc.*

So far as I have been able to learn, no author has described in the eggs of Coleoptera a structure in any way corresponding to the pole-disc. Wheeler (1889) failed to note its presence in *Leptinotarsa;* Lecaillon (1898) makes no mention of it in the chrysomelid beetles he studied (see historical part), although in several species "cellules sexuelles" (pole-cells) were found at the posterior end of the egg. In *Hydrophilus piceus* no record has been made of pole-cells (Heider, 1885, 1889; Deegener, 1900), and, since the primitive germ-cells in this beetle appear at a much later period of development, we should not expect to find a posterior pole-disc in this series. Friederichs (1906) distinguished the primordial germ-cells in *Donacia* shortly after the blastoderm was formed, but found no pole-disc. Saling (1907), who likewise fails to mention these granules, in *Tenebrio,* could not discover as early a segregation of germ-cells as has been found in species of the Chrysomelidæ.

Several other orders of insects have received more attention from embryologists than the Coleoptera, but in only two species, both Diptera, have structures similar to the pole-disc been described. In *Chironomus* Ritter (1890), after giving a brief sketch of the polar bodies and male and female pronuclei, thus continues: "In dem nächsten Stadium sind in dem Dotter keine Zellen mehr zu sehen; dagegen tritt an demjenigen Pol, an welchem später die Polzellen erscheinen, also an dem hinteren, ein eigenthümlicher wulstartiger Körper auf, welcher durch das Hämatoxylin sehr dunkel gefärbt wird. Er erscheint auf mehreren Schnitten und stellt eine etwas nach oben vorgewölbte Platte dar, welche vielfach runde Fortsätze zeigt und aus feinkörnigem Protoplasma besteht. Er bleibt bis zum Austritt der Polzellen an derselben Stelle. Da auf diesem Stadium im Inneren des Dotters keine Zelle mehr zu sehen ist, so kann man nicht umhin anzunehmen, dass dieser Körper den ersten Furchungskern enthält; die dunkle Färbung verhindert aber, dass man denselben mit Sicherheit erkenne.

"Es ist offenbar, dass nach der Theilung des Furchungskernes die Theilprodukte theils in dem dunklen wulstförmigen Körper verbleiben, theils aus demselben herausrücken." Ritter then gives a fragmentary account of the early cleavage divisions of the egg nucleus, at the end of which, the two first pole-cells appear each to contain a "grossen Kern und um denselben herum kranzförmig einen Theil des obengenannten dunklen wulstförmigen Körpers." This author maintains that the "wulstförmige Körper" is intimately concerned with the differentiation of germ-cells from somatic-cells;

he calls this structure the "Keimwulst," a term which had been used by earlier authors to designate very different organs (*e. g.*, Zaddack, 1854), and is too broad in its significance to be appropriate for the pole-disc.

A decade later Noack (1901) figured a similar structure "Polplatte," at the posterior end of the egg of another Dipteron, *Calliphora,* which he designated as the "Dotterplatte." This author holds that at the time the pole-cells appear all the nuclei in the egg are similar. "Es scheint auch thatsächlich die Platte am hinteren Pol die einzige Ursache zur ersten Zelldifferenzirung zu sein." Contrary to what is found in *Calligrapha* this "Dotterplatte" appears in *Calliphora* to impede the progress of the nuclei that encounter it. "Im nächsten Stadium haben die Kerne eine runde Gestalt angenommen, die Platte hat sich in so viel Theile getrennt, als Kerne in ihren Bereich eingetreten sind, und bildet nun um jeden dieser Kerne einen peripher gelegenen feinkörnigen Halbmond. Hiermit ist die erste Zelldifferenzirung eingeleitet." Those cells which now contain granules from the "Dotterplatte" are recognized as pole-cells, while the remaining cells which have reached the periphery of the egg constitute the blastoderm. The "Halbmond" of granules which surrounds the nucleus of each pole-cell now "schliesst sich allmählich zu einem Kreise, welcher um so mehr auffällt, weil die von ihm eingeschlossene und den Kern einbettende Protoplasmamasse fast farblos erscheint (Fig. 25 u. 26, *pz.*). Bei der Fortentwicklung der Polzellen schwindet allmählich die scharfe Grenze zwischen Zellprotoplasma und Polplatte. Letztere löst sich auf und es entsteht eine gleichmässige Pigmentirung, welche den Polzellen noch auf lange Zeit ein ganz charakteristisches Aussehen verleiht." Concerning the nature of this "Dotterplate" Noack says "dass die Platte am hinteren Pole des *Musciden-Eies* sich aus Dotterelementen zusammensetzt. Sie scheint den Zweck zu haben, das Wachsthum am hinteren Pol zu beschleunigen, ferner durch Eintritt in die Polzellen es diesen zu ermöglichen, sich auch weiterhin lebhaft zu vermehren, obgleich sie vom Dotter her keine Nahrung mehr erhalten. Schliesslich verursacht sie die charakteristische Pigmentirung dieser Zellen."

Granular inclusions have also been found in the eggs of other insects, but show only a remote resemblance to the pole-disc granules of *Calligrapha.* Thus Blockmann (1887) discovered a number of bacteria-like rods in the undeveloped eggs of *Blatta germanica.* These rods multiplied by division and were considered true symbiotic bacteria. The same author later described similar "Stäbchen" in several species of Hymenoptera (*Camponotus ligniperdus* and *Formica fusca.* The eggs of *Periplaneta orientalis* and *Ectobia livida* also contain accumulations of "bacterienartige Stäbchen" which later sink into the yolk and disappear (Heymons, 1895).

At the present time, the nature of the pole-disc granules is unknown; one of the two authors quoted above (Ritter, 1890, in *Chironomus*) claims that the pole-disc ("Keimwulst") consists of protoplasm, while the other (Noack, 1901, in *Calliphora*) considers it ("Dotterplatte") to be formed of yolk elements. I hold that the disc is probably derived from the nucleus during the growth of the oögonia.

The origin of these granules may be difficult to determine, although it seems not an impossible undertaking. Lack of material has prevented me from tracing them in stages earlier than eggs of *Leptinotarsa* just previous to laying, at which time the polar bodies are being formed (Fig. 2); here, however, the pole-disc is as large as in later stages of development and it could doubtless be found in younger eggs.

A number of facts discovered in other invertebrates may be mentioned in favor of the nuclear origin of the granules of the pole-disc. Blochmann (1886), Stuhlman (1886) and others have described for various species of Hymenoptera a budding of the nucleus in the oöcytes; these buds result in the formation of many small "nuclei" ("Nebenkerne," Blochmann; "Dotterconcretionen," Stuhlmann) each containing small dark-staining granules. The "nuclei" thus derived from the nucleus of the oöcytes pass out to the periphery of the cell and are lost to view. No pole-disc has been recorded in any of these species of Hymenoptera, but in the Dipteron, *Musca vomitoria,* where a pole-disc probably does occur, Korschelt (1886) has described bodies in the oöcytes similar to the "Nebenkerne" of

Blochmann. It is highly probable that a similar expulsion of nuclear material and corresponding decrease in the size of the germinal vesicle takes place in the oöcytes of Chrysomelid beetles, and that the particles of chromatin thus set free gather at the posterior end of the egg to form the pole-disc.[1]

Wheeler's (1897) theory to account for the presence of dark-staining granular inclusions within the eggs of *Myzostoma glabrum* suggests that the granules of the pole-disc may be derived from the nuclei of the nurse-cells which, in many insects, pass into the early oöcytes.

The results that Häcker (1897) obtained from a study of the "Keimbahn" in *Cyclops* also point to a nuclear origin of the pole-disc granules. According to this author "Aussenkörnchen" arise at one pole of the first cleavage spindle; these are derived from disintegrated nucleolar material and are attracted thus to one pole of the spindle by a dissimilar influence of the centrosomes. During the first four cleavage divisions the granules are segregated always in one cell; at the end of the fourth division these "Aussenkörnchen" disappear, but the cell which contained them can be traced by its delayed mitotic phase into the primitive germ-cells. The "Aussenkörnchen" found in the germ-cell antecedents of *Cyclops* show a remarkable resemblance to the pole-disc granules of *Calligrapha;* one important difference, however, may be pointed out, *i. e.,* the fact that in the former the granules arise from the nucleolar material of both male and female pronuclei, after, or during their conjugation, while in Chrysomelid beetles the pole-disc is already fully formed before fertilization.

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[1] Since this paper was sent to press an account of the origin of the primordial germ-cells in some parasitic Hymenoptera has appeared which furnishes proof of the nuclear origin of a structure similar to the pole-disc. I refer to the work of F. Silvestri, entitled *Contribuzioni alla conoscenza biologica degli imenotteri parassiti,* published in the *Bollettino del Laboratorio di zoologia generale e agraria* della R. Scuola Superiore d' Agricoltura di Portici, Vol. 3, April, 1908. The following quotation gives some of the results of his study of *Ageniaspis fuscicollis.* "Che il nucleolo durante la formazione dei globuli polari si conserva immutato nella parte posteriore dell' ovo e che passa intero ad una delle prime due cellule di segmentazione. Tale nucleolo, come nel *Litomastix truncatellus,* ha un' azione ritardatrice della divisione della cellula, in cui si trova ed è da ritenersi, per quanto ho anche osservato nello sviluppo delle due specie, di cui appresso tratto, un determinante della celulle germinali" (p. 53).

Another interesting variation in the origin of the germ-cells was described by Boveri (1892) in *Ascaris.* After the first cleavage division of the egg of this Nematode one cell preserves its chromosomes intact, while the other casts off the swollen ends of its chromosomes into the cytoplasm. During the first five cleavage divisions, one cell retains the two full chromosomes, while all the others contain the reduced amount. At the thirty-two cell stage, the cell containing the two full chromosomes is given up entirely to the production of the reproductive organs; the other cell gives rise only to somatic tissue.

The value of the pole-disc in the development of the insect egg can only be surmised. Ritter (1890) maintains that the first cleavage nucleus is hidden among the granules of the "Keimwulst," and he is inclined to believe that the first cleavage division separates the primitive germ-cell substance from the somatic material, although he could not demonstrate this. Noack (1901) suggests that the yolk elements of the "Dotterplatte" may hasten the growth at the posterior pole of the egg, and that later they may possibly increase the vigor of the pole-cells. That the pole-cells need special means of nourishment is doubtless the case, for contrary to the condition in the blastoderm-cells they are, at an early period, entirely separated from the yolk, and later use up energy in their migration.

Primary pole-cells are evidently characterized by the presence of yolk material, as may be illustrated by the following citations. In *Chironomus nigro-viridis* Weismann (1863) found four oval nuclei lying in the "Keimhautblastem" at the posterior end of the egg; each of these, he says, "besassen einen kreisrunden, klaren, etwas röthlich schimmernden Kern, und in einigen Lagen ausserdem noch ein oder zwei Dotterkörnchen." These are the "Polzellen." In another Dipteron, *Simula sp.,* Metschnikoff (1866) records four or five pole-cells which "bestehen ausser einem Kerne noch aus einer die feinsten Dotterkörnchen enthaltenden Zellsubstanz." The same author (1866) also states that when the pseudovum in the pædogenetic larva of *Miastor* contains twelve to fifteen nuclei, "Man bemerkt zunächst, dass der am spitzen Pole des Pseudovums liegende Keimkern von einer dicken dunkeln Dottermasse schärfer umgeben wird und mit dieser zusammen bald in eine besondere, 0.017 mm. grosse, membranlose Zelle sich abschnürt." This gives rise to the pole-cells. Schwangart (1905) found that the primitive germ-cells of *Endromis* "sind grösser und dotterreicher" than the blastoderm-cells.

The theory that the yolk contained in the germ-cells is transformed by them into the energy of motion is strengthened by the fact that the migrating germ-cells of many species of vertebrates also contain yolk-globules (Beard, 1902, in Elasmobranchs; Eigenman, 1892, in Teleosts; Nussbaum, 1880, in Amphibia; Allen, 1906, in Reptilia).

## 2. *The Migration of the Primitive Germ-Cells in the Insecta.*

### A. *The Migration of the Pole-Cells through the Pole-Cell Canal.*

The authors who first described the pole-cells of insects (Robin, 1862; Weismann, 1863) supposed that they took part in the formation of the blastoderm. This interpretation was corrected by Metschnikoff (1865) and Leuckart (1865) who maintained that the pole-cells develop into the germ-gland. These two authors, however, did not tell us how the primitive germ-cells got back into the egg after their complete separation at the posterior end. Balbiani (1882-5) added the evidence necessary to prove that the pole-cells really become the reproductive organs, but he was unable to determine whether they force their way through the blastoderm, or pass into the egg by way of a clear space left for their entrance. Sections of the eggs of *Chironomus* led Ritter (1890) to believe that the pole-cells move of their own accord through a gap in the blastoderm which then closes after them.

Escherich (1900) in *Musca* made the first accurate study of the passage of the pole-cells from the posterior amniotic cavity into the embryo. Many of his figures show conclusively not only "die Wanderung der Polzellen durch die Keimplatte" but also an "intercellular

Canal der den Polzellen den Durchtritt ermöglichte." In his Fig. 55 several pole-cells are seen lying in the groove outside of the germ-band, several are present within it and a number are represented half way through the opening in the "Entoderm," the "Polzellencanal." This migration of the pole-cells extends over a considerable period. Escherich claimed that since the region of the ventral plate containing the "Polzellencanal" later constituted a part of the "ventrale Wand des Urdarmes, . . . somit ist es uns dadurch möglich, die Mittelplatte als erste Anlage des Entoderm zu erkennen." In the last embryonic stage examined by this author, all of the pole-cells had not yet completed their migration.

Noack (1901), in *Calliphora,* also records the migration of the pole-cells from the dorsal groove of the germ-band into the embryo. This author, contrary to what Escherich found, could not satisfy himself that a definite canal exists, but decided that the pole-cells during their migration produce an adventitious gap in the "Entoderm." Noack followed the history of the pole-cells until they became scattered among the entoderm cells within the embryo.

Lecaillon (1898) is the only author who has described pole-cells in the Coleoptera. He states that after the "cellules sexuelles" are separated from the posterior end of the egg they "ne restent d'ailleurs pas longtemps en dehors de la masse ovulaire; à mesure que la segmentation progresse et que l'enveloppe blastodermique se complète, elles commencent à rentrer dans l'œuf. Pour cela, elles refoulent le vitellus devant elles et s'insinuent entre les cellules périphériques du pôle postérieur de l'œuf, au fur et à mesure que celles-ci émergent de la masse vitelline." When the blastoderm is finally completed, the "cellules sexuelles" for a group "entre le vitellus et l'enveloppe blastodermique," as indicated in his Fig. 10. In only one of the species studied, *Gastrophysa raphani,* did a variation from this order of events occur; in this form the "cellules sexuelles," although tending to re-enter the egg, remain outside, as we have found the pole-cells to do in *Calligrapha* and *Leptinotarsa.* Their migration was not carefully followed by Lecaillon in *Gastrophysa.* He found them lying at the end of the posterior amniotic cavity, and shows two of them in his Fig. 22, "dans le sillon profond qui se trouve sur le milieu de sa paroi interne;" these "pénètreront au milieu des cellules mésodermique au moment où le sillon se fermera." No pole-cell canal is figured, nor does he mention in the text by what means this penetration takes place.

Although Friederichs (1906) did not observe the formation of pole-cells in *Donacia crassipes* he found a group of germ-cells lying just within the egg at the posterior pole; these, he thinks, are derived from the blastoderm. Beneath this group is an opening in the blastoderm similar to the pole-cell canal found in *Calligrapha,* and one cannot but suspect that pole-cells arise in *Donacia* as in other Chrysomelidæ, but were overlooked by this author. The opening in the blastoderm is considered the blastopore. Friederichs says of it, "Der Blastoporus der Chrysomeliden liegt am hinteren Pol und wird verschlossen durch die Genitalanlage, welche an dieser Stelle bereits ensteht, sobald das Entoderm (die primären Dotterzellen) und das primäre Ektoderm gesondert sind. Der Blastoporus wird später zum After des Insekts."

In *Calligrapha* and *Leptinotarsa,* a definite pole-cell canal is present, homologous to the "Polzellencanal" found by Escherich in *Musca.* The origin of this canal has been described in a previous chapter (III) and the progress of the pole-cells through it was there followed in detail. It undoubtedly is the opening in the blastoderm caused by the pole-cells during their formation; this opening is kept free from cells by a plug of cytoplasm connecting the group of pole-cells lying in the posterior amniotic cavity with the pseudoblastodermic nuclei just within the egg (Fig. 25). The canal closes only after all the pole-cells have passed through it (Fig. 40). It will be shown later that in *Calligrapha* the pole-cells migrate into the embryo by means of amœboid movements.

### B. *The Migration of the Germ-Cells within the Embryo.*

It is difficult to ascertain in many cases whether the germ-cells described by the earlier authors changed their position within the embryo by means of an active migration, or were passively moved about by the shifting of the other embryonic tissues. Metschnikoff

(1866), Balbiani (1885) and Ritter (1890), in the Diptera, and Huxley (1858), Metschnikoff (1866), Balbiani (1866), Witlaczil (1884) and Will (1888), in the Hemiptera, describe a precocious segregation of the germ-cells. In the Diptera no active migration is reported after the pole-cells re-enter the egg; in the Hemiptera no movements have been noted at any stage in the history of the germ-cells.

The primitive germ-cells of several species of Orthoptera are, when first seen, in the act of penetrating the walls of the cœlomic cavities (*Blatta,* Cholodkovsky, 1891, and Heymons, 1891; *Xiphidium* Wheeler, 1893). As noted elsewhere in this paper, Heymons (1895) has established a much more extensive migration of the germ-cells in *Blatta* and other allied forms than was described by the authors named. A migration similar to that found in *Blatta* occurs in *Forficula* (Heymons, 1895).

The migration of the germ-cells in the Aptera has been recorded by two observers, Heymons (1897) and Claypole (1898). The former found that the germ-cells in *Lepisma* have an ectodermal origin at the posterior end of the germ-band. They soon "sich zerstreuen und einzeln, zwischen und neben den Mesodermzellen nach vorn wandern." The germ-cells are less favorable for study in *Lepisma* than in *Periplaneta,* but Heymons nevertheless convinced himself "dass die Wanderung der Geschlechtszellen sich ganz ähnlich wie bei den Orthopteren vollzieht." According to Claypole (1898) the germ-cells of *Anurida* move into the yolk at a late embryonic stage and begin to mingle with its globules. Under high magnification "the peculiarly 'succulent' character of the cells" could be seen.

Carriere and Burger (1897) describe in the Hymenopteron, *Chalicodoma,* the migration of the germ-cells from the third and fourth abdominal segments into the fifth.

In the "drone eggs" of the bee, the germ-cells penetrate the walls of the primitive somites and congregate in the cœlomic cavities. (Petrunkewitsch, 1903).

Although Woodward (1889) correctly observed the place of origin of the germ-cells in the Lepidopteron, *Vanessa,* it remained for Schwangart (1905) to follow their further history. In *Endromis* the germ-cells separate into groups which migrate from a point near the posterior end of the egg, to the fourth, fifth, and sixth abdominal segments.

As soon as the pole-cells of *Calligrapha* have passed through the pole-cell canal, they loose their pronounced pseudopodia-like processes and become nearly spherical (Fig. 55); nevertheless, they undergo a decided change in position. They move away from the inner end of the pole-cell canal, and creep along between the yolk and the germ-band (Figs. 47-49). Thus two groups are formed near the developing cœlomic sacs; each group probably contains an equal number of cells. The smallest number I have counted in one group at this time (Stage M) is thirty; the largest number, thirty-four. As there is some difficulty in obtaining an accurate count, it seems probable that the sixty-four germ-cells are equally divided and that each germ-gland receives thirty-two. Some of the germ-cells migrate not only laterally along the germ-band but also back toward the posterior end of the egg, where we find them forming narrow strands in the last abdominal segments (Figs. 39-41). From this stage on, the germ-cells are not very active; they move closer to one another to form the compact germ-glands. I was unable to determine whether the later movements of the germ-cells are due to an active migration, or to the tensions created by the growth of the surrounding tissues; the latter seems the more probable.

### C. *The Method of Locomotion of the Germ-Cells.*

Nearly all of the authors, who have observed the migration of the germ-cells in the Insecta, have failed to describe their method of locomotion. Thus Robin (1862) and many of his contemporaries state that in the Diptera, the pole-cells, shortly after their appearance, move back into the egg. These authors, however, give no explanation to account for these movements, and one is not enlightened as to whether the pole-cells are passively carried from place to place, or whether they undergo an active migration. That amœboid movements might possibly explain the re-entering of the egg by the pole-cells, was first suggested by Weismann (1882). He was unable to follow the history of these cells in *Chironomus,* because they disappeared from view beneath the egg. One cell lying near the anterior end of the egg was capable of amœboid movements, but its identity was not established. Ritter (1890), after finding that the pole-cells in *Chironomus* penetrate the blastoderm, concludes that "anders als durch aktive Bewegung können sie wohl kaum in die Lage kommen, in welcher wir sie Fig. 10 finden." The cells in the figure mentioned have, however, a spherical form exhibiting no pseudopodia-like processes.

Turning now to the Coleoptera, we find sufficient evidence to prove that the pole-cells migrate by means of amœboid movements. Although Wheeler (1889) failed to find the pole-cells in the very early stages of *Leptinotarsa,* he figures several of them (his Fig. 82) in a sagittal section of an egg carrying a segmented germ-band. Here are shown three cells "which are on the surface of the embryo in the amniotic cavity. They are very large and clear and the more anterior is apparently creeping in the manner of an Amœba, along the surface of the abdominal ectoderm. These cells, the ultimate fate of which I have been unable to determine, probably escape from the anal orifice of the gastrula before it closes." This author also shows in transverse section (his Fig. 87) a cell which, he says, is "about to wander through the blastopore into the amniotic cavity." He suggests that this may be the homologue of the "Polzellen." My sections prove that these are really pole-cells and that they creep along the surface of the ectoderm by amœboid movements, but the direction of their migration is the reverse of that stated by Wheeler, *i. e.,* they are not wandering outward into the amniotic cavity but are on their way into the embryo.

In *Clytra* and other species of Chrysomelid beetles, Lecaillon (1898) finds that the "cellules sexuelles," as he designates the pole-cells, migrate back into the egg shortly after their appearance. In another species (*Gastrophysa raphani*) studied by this author, these cells remain outside of the egg until a later stage of development, and then they penetrate the "ectoderm." Lecaillon does not present any evidence to account for this migration, he says, however, that these cells "se montrent en général moins bien fixées que les autres cellules." In my material no difficulty was experienced in obtaining perfectly fixed pole-cells, and I conclude that Lecaillon was deceived by the irregular outline of the "cellules sexuelles," and that in Clytra the apparent distortion of these cells was due, not to poor fixation, but to their amœboid character.

Several authors have described the locomotion of primitive germ-cells in other orders of insects. Ayers (1883) states that the germ-glands of *Oecanthus* "are first seen as two irregular groups of amœboid cells." In *Forficula* (Heymons, 1895) the germ-cells arise near the posterior end of the egg and migrate anteriorly. "Die Bewegung dürfte hierbei durch Aussenden amöboider Fortsätze erfolgen, die man jetzt an den Zellen gar nicht selten beobachten kann."

Heymons (1895) says of *Periplaneta,* "Aehnlich wie in gewissen Stadien von *Forficula* scheint die Fortbewegungsart der Zellen hierbei eine amöboide zu sein, es kann dies wenigstens aus den zahlreichen Gestaltsveränderungen der Geschlechtszellen geschlossen werden, die bald rundlich, bald langgestreckt sind oder lappige Fortsätze aussenden."

The pole-cells of both *Calligrapha* and *Leptinotarsa* not only migrate by their own activity, but their movements are distinctly amœboid. It has been noted above that every preblastodermic nucleus has long cytoplasmic processes extending out on all sides into the yolk. It has also been shown that these processes become blunt in the case of the pole-cells when separation from the egg takes place (Fig. 22). If we examine the pole-cells from the time they are protruded until they become aggregated into a distinct germ-gland, we discover a series of stages which establish their amœboid character as well as it is possible to do in fixed material.

During their protrusion, the pole-cells have still an irregular outline, but their cytoplasmic processes are no longer present on their outer surface; this is probably due to being pressed against the "Keimhautblastem" (Figs. 20 and 21). After complete separation, however, they again acquire an amœboid shape, their blunt pseudopodia containing most of the granules taken from the pole-disc (Fig. 22). This appearance is retained until the pole-cells begin to migrate through the pole-cell canal; then the pseudopodia are no longer found on all sides of the cell, but are definitely directed toward the entrance to the canal. This may be seen in a transverse section of an egg of *Leptinotarsa* similar to Stage H (Fig. 35). In this figure two pole-cells are near the inner end of the pole-cell canal, two are creeping along one side of the groove in the germ-band, while three others are still in the amniotic cavity, evidently moving toward the canal. A greater magnification brings out more clearly the shape of the pole-cells and the direction of their migration. Fig. 56 shows two of these cells taken from a transverse section (Fig. 38) of the tail-fold of an embryo similar to Stage J. The pseudopodia are here unmistakable; they are extended toward the entrance to the pole-cell canal. The hyaline cytoplasm at the tips of the pseudopodia resembles the ectoplasm of Amœba; it will be recognized as the vacuolated layer which was carried away from the "Keimhautblastem," where the pole-cells were extruded from the egg (Fig. 21, vac. st.). The pole-cells seem to be thigmotactic, few of them being found free in the amniotic cavity; they are usually observed close to the germ-band or crowded one against another.

Fig. 55 shows a pole-cell and an adjacent blastoderm-cell enlarged from the sagittal section shown in Fig. 37. Here we find little or no evidence of pseudopodia. This is the usual condition of the pole-cells after they have reached the interior of the egg. Their method of progression from this stage on is not easily made out. In a later stage (Stage M) the germ-cells are partly surrounded by mesoderm-cells; their outline is still irregular, as is shown in the enlarged drawing (Fig. 57), but no long pseudopodia such as are present in the younger embryos can be seen. It may be that the pole-cells cease to move actively after they reach the interior of the embryo, and that they are pushed into place by the rapidly proliferating mesoderm-cells.

### 3. *The Origin and Early History of the Germ-Cells in the Insecta.*

No general statement can be made regarding the time and place of origin of the primitive germ-cells in the Insecta, as the species which have been examined represent only a small proportion of the types necessary for a thorough understanding of this subject.

Petrunkewitsch (1901 and 1903) has described the origin of the primitive germ-cells in the "drone eggs" of the bee at a period earlier than has been recorded for any other insect. The inner half of the first polar body of these unfertilized eggs unites with the second polar body to form the "Richtungscopulationskern" which is the primordial germ-cell. Weismann (1904) vouches for the exactness of Petrunkewitsch's results. In other Hymenoptera no germ-cells have been found previous to the appearance of the mesoderm (Carriere and Burger, 1897).

The primitive germ-cells (pole-cells) of several species of Diptera have a very early origin. Weismann (1904), discussing the development of the reproductive cells in connection with his "germ-plasm theory," says: "If we could assume that the ovum, just beginning to develop, divides at its first cleavage into two cells, one of which gives rise to the whole body (soma) and the other only to the germ-cells lying in this body, the matter would be theoretically simple. . . . As yet, however, only one group of animals is known to behave demonstrably in this manner, the Diptera among insects. . ." I have been unable to find in embryological literature any account of such a phenomenon in this order of insects. The pole-cells of Diptera are always found at the posterior end of the egg. The time of their first appearance varies in the different species described. In *Miastor* the primordial pole-cell nucleus can be distinguished when there are only eight to fifteen nuclei in the pseudovum (Leuckart, 1865; Metschnikoff, 1865). In *Chironomus* the single primordial pole-cell appears before the blastoderm is formed and is closely followed by a second, these two then divide, re-enter the egg, and develop into the germ-glands (Grimm, 1870; Weismann, 1882; Jaworowski, 1882; Balbiani, 1885; Ritter, 1890). The first pole-cell nucleus in this species may divide before it separates from the egg. Weismann (1863) noted four pole-cell nuclei lying in the "Keimhautblastem," while several authors have described the appearance of two, before separation takes place (Grimm, 1870; Weismann, 1882).

In other species of Diptera the primordial pole-cell nucleus apparently divides several times before it reaches the surface of the egg; there are fifteen to twenty in *Calliphora* (Noack, 1901), and four to five in *Simula* (Metschnikoff, 1866) and in *Pulex* (Packard, 1872), although neither of the latter was examined carefully.

Pole-cells are also found in several Chrysomelid beetles. Lecaillon (1898) made no attempt to count the number of "cellules sexuelles" in *Clytra*, but states that they are the cells which first reach the "Keimhautblastem" at the posterior end of the egg. In *Calligrapha* I have shown that the primitive pole-cell nuclei may be recognized when they are four in number, but that these divide twice before they separate from the egg, *i. e.*, there are sixteen which pass through the pole-disc. After separation these divide twice, giving rise to sixty-four. This number remains constant until the embryo is nearly ready to hatch (Fig. 45); then the germ-cells increase rapidly by mitosis.

The very early stages of pole-cell formation were not observed by me in *Leptinotarsa*. When the pole-cells were first seen in this species, they formed a group lying at the posterior end of the egg (Fig. 26); they are in every way similar in appearance to those found in *Calligrapha* (Fig. 24, Stage B), being amœboid in shape and containing a layer of granules which they have gathered from the pole-disc (Fig. 2). Embryos similar to Stages A to O were examined and in every case the germ-cells were discovered occupying a position which corresponds almost exactly to that found in *Calligrapha*. Wheeler (1889), in his work on *Leptinotarsa*, not only failed to find the pole-disc, but also overlooked the pole-cells at the posterior end of the egg. His Figs. 66, 67 and 70 represent surface views of embryos like my Stages C, D and F, of *Calligrapha*. The group of pole-cells is present in every one of these stages in *Lepinotarsa*, and I cannot understand why Wheeler failed to find them. On page 321 Wheeler (1889) says: "Sections taken in all directions through the egg show the blastoderm to be of even thickness over the whole surface (Fig. 63)." This is not true of the eggs of *Leptinotarsa* I have examined, as there are two or three layers of cells at the posterior end at the stage to which he refers. The space beneath the pole-cells remains free from blastoderm-cells, and later becomes the pole-cell canal just as we found in *Calligrapha*.

The primitive germ-cells may be recognized in the parthenogenetic eggs of Aphids shortly after the blastoderm is completely formed. Some authors were able to trace them back to a single cell which separates from the inner surface of the blastoderm near the posterior end of the egg (Balbiani, 1866; Witlaczil, 1884; Will, 1888).

The only investigators who have recorded an early appearance of the primitive germ-cells in the Leptidoptera are Balbiani (1869-72), Woodworth (1889) and Schwangart (1905). These authors found a thickening of the blastoderm near the posterior end of the egg. The inner cells of this thickening differentiate into germ-cells; later these migrate singly into the fourth to the eighth abdominal segments (Schwangart, 1905).

The foregoing accounts show that those embryologists who hold that the germ-cells in the Insecta have a mesodermal origin are not in harmony with the results obtained by recent investigators. Heymons (1891), Korschelt and Heider (1892), and Wheeler (1893) all regarded the primitive germ-cells of Diptera and Hemiptera "as derived by a process of precocious segregation from metameric gonads like those of the Orthoptera" (Wheeler, 1893). The germ-cells in the Orthoptera (*Blatta* and *Periplaneta*), however, do not arise metamerically from the mesoderm and later migrate into the primitive somites (Heymons, 1895).

As noted above, the stage of embryonic development in which the germ-cells can first be recognized varies considerably in different species of insects. By the majority of authors the reproductive cells have been considered of mesodermal origin, by others they are supposed to arise from ectoderm-cells, blastoderm-cells, yolk-nuclei, or early cleavage nuclei. I believe with Woodworth (1889) that "the germinal cells do not belong to any layer, but are the products of the first divisions of the egg cell; they take part generally in the formation of the blastoderm and then migrate into the mesoderm. . . . In all cases where they are supposed to come from the mesoderm, the later stages comparatively are the only ones known." Heymons (1893) four years later was led to similar conclusions. This author states "dass die Geschlechtszellen der Insekten überhaupt nicht von diesem oder jenem 'Keimblatte' abzuleiten sind, sondern nur scheinbar je nach dem Zeitpunkt ihres Hervortretens bald dieser, bald jener Zellenschicht angehören.

"Wenn auch die Trennung zwischen somatischen Zellen und Geschlechtszellen bei den meisten Insekten erst spät bemerkbar wird, so werden wir somit doch annehmen müssen, dass ein solcher Unterschied bereits vom Beginne der Entwicklung an vorhanden ist.

"Es mag noch hervorgehoben werden, dass die Geschlechtszellen der Insekten nicht, wie man bisher geglaubt hat, in metamerer Anordnung in den einzelnen auf einander folgenden Abdominalsegmenten zur Anlage kommen, sondern dass ihr Ursprung am hintersten Ende des Keimstreifs zu suchen ist, von wo aus erst im Laufe der Entwicklung eine Wanderung oder Verschiebung nach vorn hin erfolgt. Dies trifft zunächst für die hier beschriebenen Formen zu, hat möglicherweise aber für sämmtliche Insekten Gültigkeit." Heymons is an investigator who has not been content to work on a few widely separated types of insects, but has made comparative studies of nearly allied species. The value of this kind of research is strikingly shown by the results he obtained from a study of *Blatta* and *Periplaneta.*

In an early paper Heymons (1891) stated that the germ-cells of *Blatta* are derived from the mesoderm just previous to the segmentation of the germ-band. Later (1895) *Periplaneta* was also examined. In this Orthopteron the germ-cells were found to arise from the ectoderm at the posterior end of the egg; they separate from one another, migrate anteriorly, and arrange themselves intersegmentally. A re-examination of *Blatta* convinced Heymons that the germ-cells originate in this cockroach as they do in *Periplaneta,* but can be distinguished from the mesoderm-cells only when they reach the primitive somites. This is made more certain by the discovery of a similar origin and migration in *Forficula* (Heymons, 1895) and *Lepisma* (Heymons, 1897).

In *Calligrapha* all the nuclei of the egg are apparently alike, potentially, until in their migration toward the surface they reach the "Keimhautblastem;" then those which chance to encounter the granules of the pole-disc are differentiated by their environment, *i. e.,* the granules, into germ-cells. In other words, whether or not a cell will become a germ-cell depends on its position in the egg just previous to the formation of the blastoderm. The cleavage nuclei of the beetle's eggs are not separated by cell boundaries, as are those of several other animals (*e. g. Cyclops* and *Ascaris*), where an earlier differentiation of the primordial germ-cells takes place, but are intimately connected by the cytoplasm which is present throughout the egg in the interdeutoplasmic spaces. We have thus a syncytium in which the nuclei are widely separated from one another by the enormous mass of yolk. The various substances (*e. g.,* the granules of the pole-disc) are, therefore, less easily segregated into a single cell in the egg of *Calligrapha* than are similar structures (*e. g.,* the "Aussenkörnchen" of *Cyclops,* Häcker, 1897) in alecithal eggs. This fact may account for the relatively late stage at which the primitive germ-cells of *Calligrapha* and allied forms can be recognized as such.

## V. SUMMARY.

1. A layer of dark-staining granules, the pole-disc, is present at the posterior end of the eggs of *Calligrapha* and *Leptinotarsa* before fertilization takes place; this layer is later intimately associated with the development of the pole-cells.

2. The genesis of the pole-cells is as follows: (1) four nuclei lying near the posterior end of the egg are recognized by their position as pole-cell antecedents (Figs. 5 and 12); (2) these four nuclei divide producing eight daughter nuclei which move closer to the periphery of the egg (Fig. 7); (3) these in turn divide resulting in sixteen nuclei, arranged in pairs, each of which separates entirely from the egg, carrying with it a portion of the "Keimhautblastem" containing pole-disc granules (Figs. 13 and 19-22); (4) the sixteen primary pole-cells divide to form thirty-two secondary pole-cells (Fig. 14); these divide resulting in sixty-four tertiary pole-cells (Figs. 15-16) which do not increase in number until a late period of embryonic life (Fig. 45); (5) in mitosis the pole-disc granules are, approximately, equally distributed between the two daughter cells (Figs. 17 and 27 *a*).

3. That area of the egg through which the pole-cells pass is not closed by the blastoderm but becomes the pole-cell canal, through which the pole-cells later migrate into the embryo (Figs. 33-40).

4. The blastoderm-cells which fail to cover this area form a syncytium containing pseudoblastodermic nuclei; these nuclei for a long period lie just within the egg near the pole-cell canal, and finally disintegrate (Figs. 24-25 and 33-39).

5. After their separation from the egg the history of the pole-cells is as follows:

(1) The pole-cells are carried slightly forward on the ventral surface of the egg by the contraction of the ventral plate (Fig. 29, Stage E); (2) they sink into the posterior depression of the ventral groove, which is the beginning of the posterior amniotic cavity (Figs. 33-34, Stages F-G); (3) they are carried along by the developing tail-fold, which penetrates dorso-anteriorly into the yolk (Stages H-K); (4) they migrate through the pole-cell canal into the embryo by means of amœboid movements (Figs. 35-40, 47, 56); (5) upon reaching the interior of the embryo they separate into two groups, which come to lie as a strand on either side of the body, in the last two abdominal segments (Figs. 40, 47-49 and Stages K-L); (6) these two strands become shorter by a crowding together of the germ-cells (Fig. 41, Stage M); (7) each of the two germ-glands thus produced acquires an epithelial covering of mesoderm-cells (Figs. 42, 50); (8) the germ-glands, situated as before in the last two abdominal segments, are carried, by the shortening of the embryo, to a ventral position on either side of the body (Figs. 42-43, Stages M-O); (9) by its lateral growth around the yolk, the embryo carries the germ-glands to a point near the dorsal surface on either side of the mid-intestine (Figs. 51-53, 43-45); (10) the sexes can be distinguished at this time by the shape of the germ-glands, that of the male being dumb-bell shaped (Fig. 45), while the female reproductive organ is pear-shaped, and shows the development of terminal filaments (Figs 46 and 53).

## VI. MATERIAL AND METHODS.[2]

The eggs of Chrysomelid beetles are usually laid on the leaves of the plants, which serve as food for the larvæ. *Callagrapha multipunctata* deposits its eggs in batches of from two to twenty on the under surface of willow leaves (*Salix longifolia*). A number of these beetles were kept in stender dishes and their eggs as soon as laid were transferred to watch glasses. All the eggs laid at one time were found to be in practically the same stage of development, and the batches were, therefore, carefully separated from one another. The age of the eggs proved to be no exact criterion of their developmental progress as external factors (temperature, humidity, etc.) play an important part in the rapidity of embryonic growth. Thus, two eggs of the same age which are kept under different environmental conditions, may on examination be found in very different stages of development. For this reason the various embryos figured are not designated by the number of hours since the eggs were laid, but are classed arbitrarily according to their stage of development.

Eggs of *Calligrapha lunata* were found on leaves of the wild rose (*Rosa blanda*); those of Leptinotarsa were taken in abundance from potato plants (*Solanum tuberosum*). The eggs were preserved at intervals of from fifteen minutes to one hour and a complete series was obtained from those just laid to those containing embryos ready to hatch. A few eggs of *Leptinotarsa* were dissected out from the oviducts of the adult beetles.

A large number of fixing fluids were tried; the one which gave the best results was a modification of Petrunkewitsch's fluid. The mixture was heated to a temperature of about 80° C., and poured over the eggs; this fluid was followed after half an hour by seventy per cent alcohol containing a small amount of iodine. After the above fixation, the chorion stood away from the egg so that it could easily be removed with needles under the binocular microscope. Sections were cut $6^2/_3$ microns thick and were stained on the slide; Mayer's acid-hæmalum followed by Bordeaux red, was used more than any other combination, although most of the commoner methods were employed in checking the results. The germ-cells stained deeply in orange G, and in picric acid, and could be distinguished without difficulty by their affinities for these colors. Entire embryos were stained in Conklin's picro-hæmatoxylin, Mayer's acid-hæmalum or Partsch's alum cochineal; some remarkably clear preparations of the posterior end of the egg were procured by overstaining in acid-hæmalum, decolorizing in absolute alcohol containing one per cent HCl, and then immediately clearing in xylol, and mounting. By this method the density of the hæmatoxylin was reduced and a transparent reddish hue remained; the granules of the pole-disc and nuclear structures could be clearly distinguished in thick preparations after this treatment.

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[2] For an account of the breeding habits of these beetles see Hegner, 1908.

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## EXPLANATION OF PLATES.

### Reference Letters.

am., amnion.
am.s., amnioserosal fold.
ap., appendage.
bl., blastoderm.
bl.c., blastoderm-cell.
bl.c.n., blastoderm-cell nucleus.
ch., chorion.
coe., coelomic cavity.
d.v., disintegrating yolk-globule.
ec., ectoderm.
e.m.i., epithelium of mid-intestine.
f.b., fat-body.
g.bd., germ-band.
g.c., germ-cell.
g.gl., germ-gland.
K.h.bl., "Keimhautblastem."
l.f., lateral fold of ventral groove.
mp.t., Malpighian tubule.
ms., mesoderm.
m.t., muscular tissue.
n.s., nervous system.
p.am.c., posterior amniotic cavity.
p.bl.n., preblastoderm nucleus.
p.c., pole-cell.
p.c.c., pole-cell canal.
p.c.n., pole-cell nucleus.
p.d., pole-disc
p.d.g., pole disc granule.
ps.bl.n., pseudo-blastodermic nucleus.
s.m., splanchnic mesoderm.
so.m., somatic mesoderm.
sr., serosa.
t.f., terminal filament.
tr., tracheal invagination.
v., yolk.
vac., vacuole.
vac.st., vacuolated stratum of the "Keimhautblastem."
vit., vitellophag.
vt. g., ventral groove.

## EXPLANATION OF PLATE I.

Unless otherwise stated all the figures on this and succeeding plates were drawn from eggs or embryos of Calligrapha multipunctata. Lines drawn through figures on this plate indicate where sections were made, and refer by number to more highly magnified illustrations on the following plates. The germ-cells (pole-cells) are represented by small rings. All are magnified 35 diameters.

*Stage A.* The pole-cell nuclei are protruding at posterior end. No blastoderm-cell nuclei have yet reached the surface.

*Stage B.* All the pole-cells (64) lie in a group outside of the egg at the posterior end.

*Stage C.* Ventral surface of egg. The lateral folds of the ventral plate have appeared.

*Stage D.* View of ventral surface. The group of pole-cells has been carried part way up on the ventral surface.

*Stage E.* Ventral view of egg. Pole-cells lie in posterior depression of ventral groove. First appearance of germ-band.

*Stage F.* Ventral groove narrower than in Stage E. Pole-cells partly covered by lateral folds.

*Stage G.* Lateral view of same egg as in Stage F. Dotted line shows depth or ventral groove. Pole-cells lie at entrance to pole-cell canal.

*Stage H.* Lateral view. Amnioserosal fold partly covers germ-band. Pole-cells lie at end of posterior amniotic cavity; a few have passed through the pole-cell canal.

*Stage J.* Germ-band, in lateral view, almost covered by amnioserosal fold. More pole-cells are inside of germ-band than in younger stage (H).

*Stage K.* View of lateral surface of segmented germ-band. Nearly all of the pole-cells are now inside the embryo.

*Stage L.* Lateral view after appearance of appendages. Tail-fold shorter than in preceding stage (K). Germ-cells have separated to form a group on either side of tail-fold in last two abdominal segments.

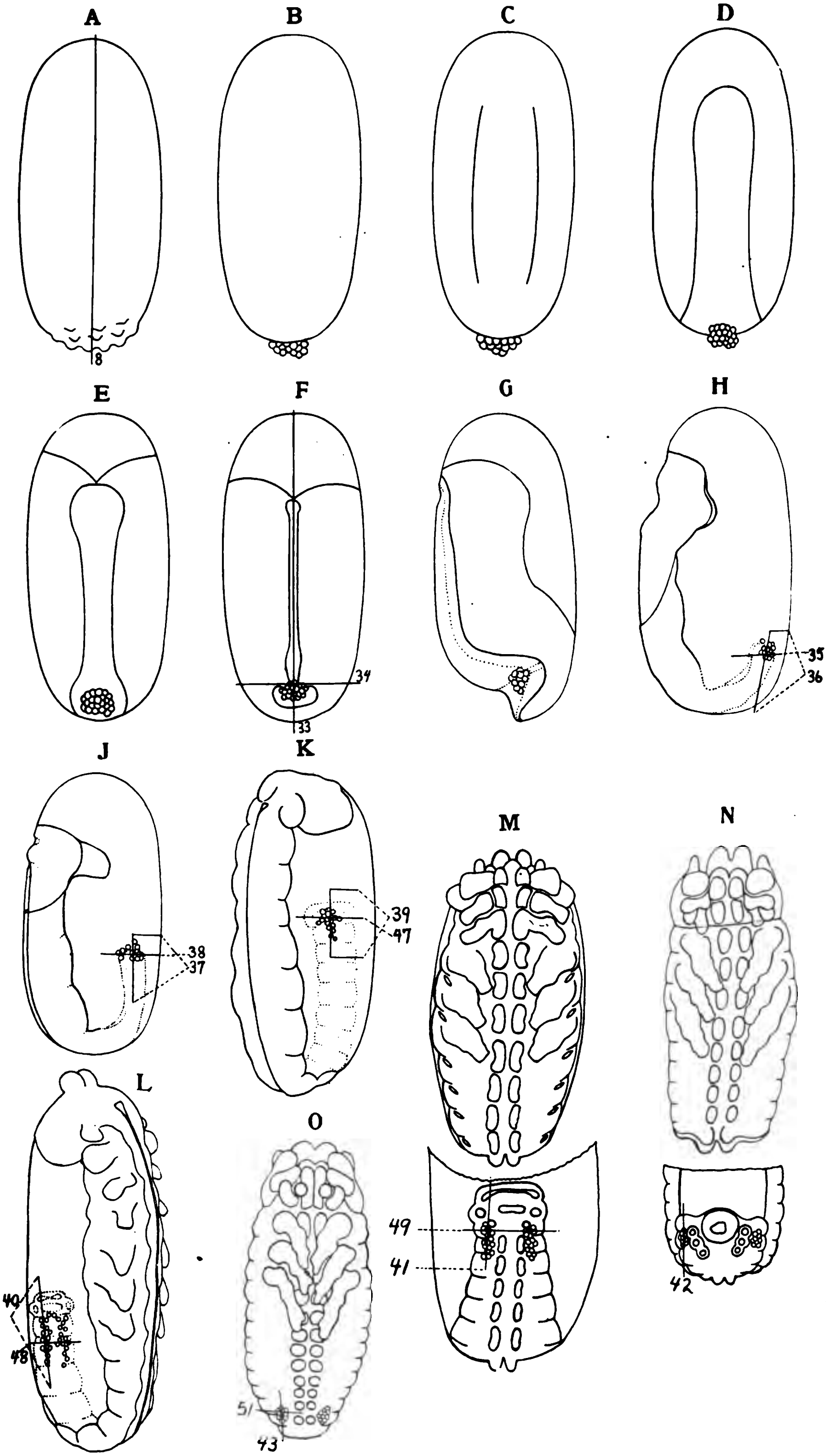
*Stage M.* Upper figure represents ventral surface of embryo; lower figure, the posterior portion on dorsal surface. Tail-fold is short and broad. Germ-cells are closer to one another than in Stage L.

*Stage N.* Upper figure represents ventral surface of embryo; lower figure, the tail-fold on the dorsal surface. Germ-cells form two definite compact germ-glands.

*Stage O.* Ventral view of embryo. End of tail-fold now coincident with posterior pole of egg. Germ-glands lie near ventral surface on either side of median line.

GERM-CELLS IN CHRYSOMELID BEETLES. PLATE I.

ROBERT WILHELM HEGNER.



## EXPLANATION OF PLATE II.

A number in brackets refers to the figure from which the cells have been enlarged.

Fig. 1. Surface view of posterior end of an egg 2½ hours after deposition. The pole-disc occupies about ⅛ of the entire area. Only those yolk-globules adjacent to the pole-disc are shown. a, circumference of egg indicated by single line. × 200.

Figs. 2 to 4 represent portions of the posterior end of eggs in longitudinal section showing the arrangement of the pole-disc granules. × 850.

Fig. 2. Egg taken from the oviduct of Leptinotarsa. Granules of pole-disc are here very close together forming a broken strand.

Fig. 3. Egg 13 hours after laying. This egg contained about 133 nuclei. Granules of pole-disc suspended in the inner stratum of the "Keimhautblastem" forming a network.

Fig. 4. Egg 11 hours after laying. Pole-disc granules widely separated from one another.

Figs. 5 to 11 represent longitudinal sections through the posterior end (except figure 8) of eggs, showing early stages in pole-cell formation. Granules of pole-disc represented by dots. × 60.

Fig. 5. One of the 4 pole-cell antecedents (a) is shown in this figure.

Fig. 6. Three of the nuclei (a) in this figure divide once giving rise to pole-cells.

Fig. 7. The three nuclei indicated at a will become pole-cells.

Fig. 8. Longitudinal section through an egg in Stage A. Seven pole-cell nuclei are protruding from the posterior end of the egg.

Fig. 9. Three of the six pole-cell nuclei represented in this figure have entirely separated from the egg.

Fig. 10. Blastoderm completed. Pole-cells form two layers.

Fig. 11. Pole-cells form an irregular group. a, pole-cell in anaphase of division.

Figs. 12 to 15 represent surface views of the posterior end of eggs, showing successive stages in pole-cell formation. × 200.

Fig. 12. The four nuclei (a) under the center of the pole-disc will give rise to all of the pole-cells.

Fig. 13. Eight pairs of pole-cell nuclei may be recognized by their dark granules. Eleven of the blastoderm-cell nuclei (a) contain a few pole-disc granules. b, strand of cytoplasm connecting a pair of pole-cells.

Fig. 14. Thirty-four pole-cells are present, each containing granules from the pole-disc. One pole-cell (17) is in the late anaphase of mitosis.

Fig. 15. Sixty-three pole-cells are visible. The blastoderm is fully formed.

Fig. 16. Lateral surface view of the posterior end of egg in Stage B. Part of the pole-cells occupy an indentation in the end of the egg. The pseudoblastodermic nuclei appear as a dark mass in the interior. × 200.

Fig. 17. Pole-cell in anaphase of mitosis. Enlarged from Fig. 14, position 17. The pole-disc granules have been equally distributed to the two ends of the cell. × 850.

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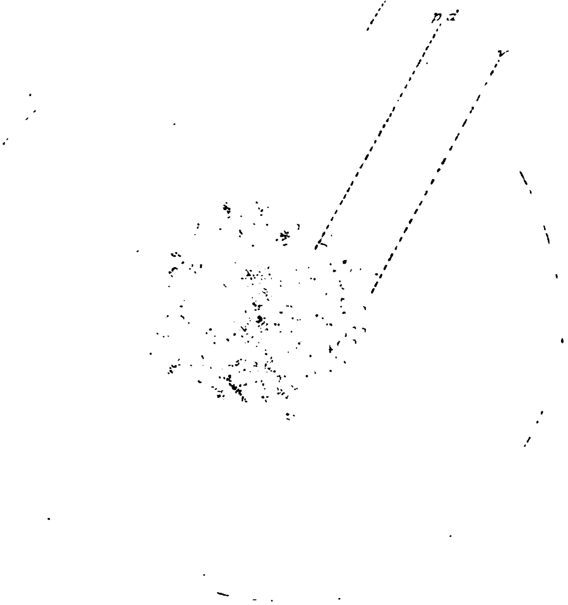

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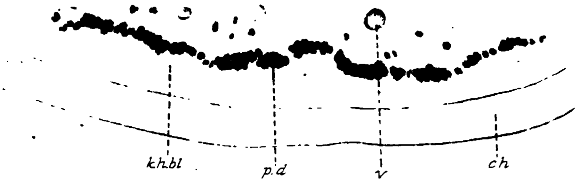

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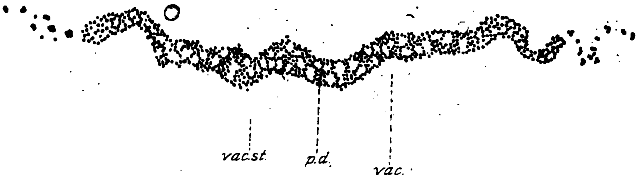

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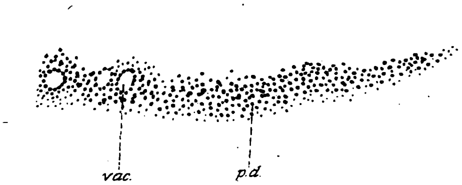

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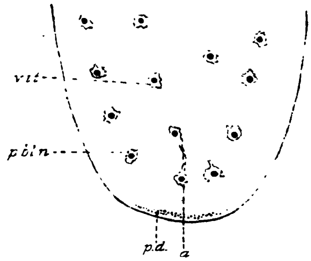

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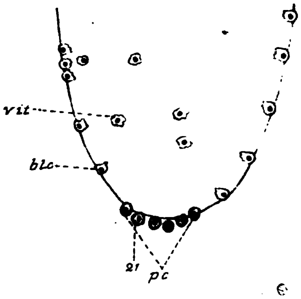

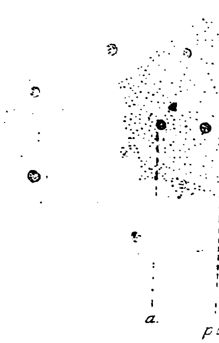

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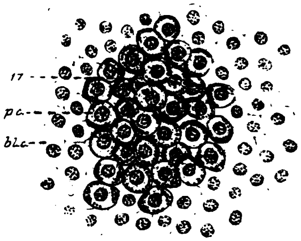

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# PLATE II

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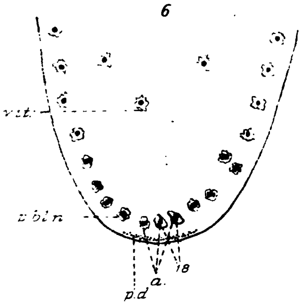

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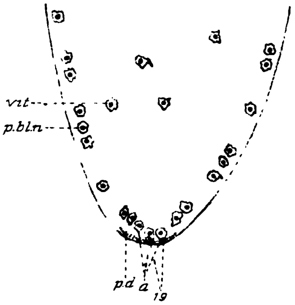

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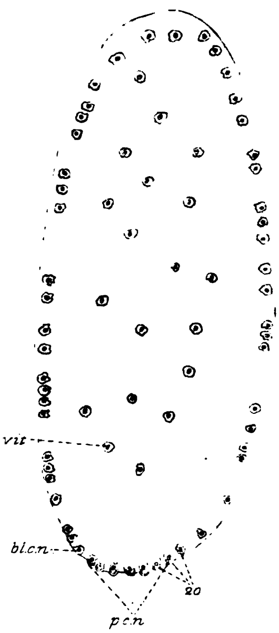

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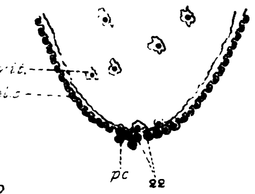

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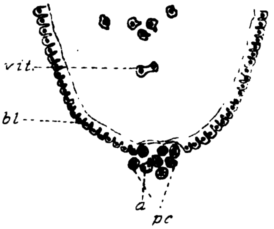

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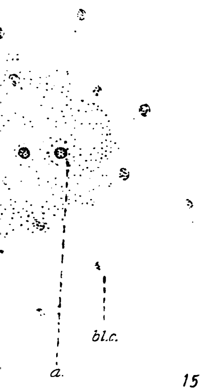

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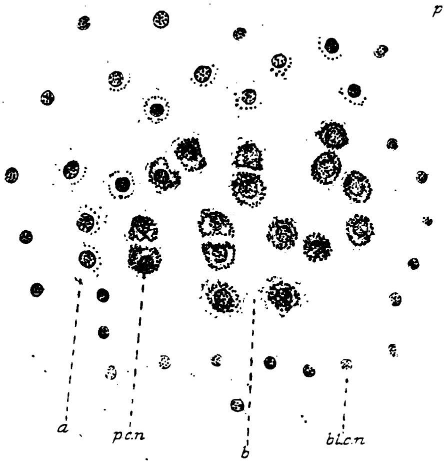

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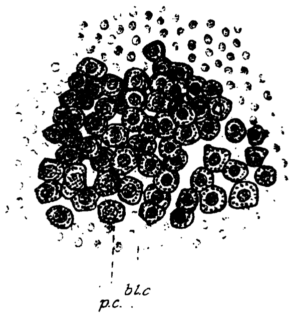

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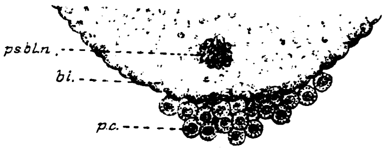

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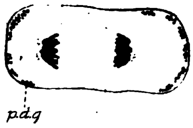

## EXPLANATION OF PLATE III.

FIG. 18. Two nuclei which will give rise to four pole-cells; enlarged from Fig. 6, position 18. a, boundary between nucleus and cytoplasm; b, chromosomes partially arranged in equatorial plate. × 850.

FIG. 19. Two nuclei which will become pole-cells; enlarged from Fig. 7, position 19. a, pole-disc granules accumulated in compact mass. Compare pole-disc in Fig. 2. × 850.

FIG. 20. Two pole-cell nuclei (a and b) and one blastoderm cell nucleus (c); enlarged from Fig. 8, position 20. d, pole-disc granules which remain in "Keimhautblastem" after the pole-cells are protruded. × 850.

FIG. 21. One pole-cell nucleus enlarged from Fig. 9, position 21. The pole-disc granules entirely surround the nucleus. × 850.

FIGS. 23 to 27 represent longitudinal sections of portions of the posterior end of eggs, showing the arrangement of the pole-cells, and the formation of the pseudoblastodermic nuclei and the pole-cell canal. × 200.

FIG. 23. Blastoderm ends abruptly where it encounters the pole-cells. a, pole-cell in anaphase of mitosis.

FIG. 24. A few blastoderm-nuclei have been pushed upward past the pole-cells into the yolk to form pseudoblastodermic nuclei.

FIG. 25. Pseudoblastodermic nuclei now form a funnel shaped syncytium just above the pole-cells.

FIG. 26. In this figure (Leptinotarsa) the pole-cells are larger and have larger nuclei than the blastoderm-cells. A few pseudoblastodermic nuclei (ps.bl.n.) are present.

FIG. 27. The pole-cells in C. lunata here show an arrangement similar to those in C. multipunctata (Fig. 24). a, one pole-cell in the last stage of division.

FIG. 28. A pole-cell and an adjacent blastoderm-cell enlarged from Fig. 25, (28). × 850.

FIGS. 29 to 32 represent surface views of eggs showing how the group of pole-cells is carried into the posterior amniotic cavity. × 50.

FIG. 29. Ventral view (Stage E). A shortening of the germ-band has carried the pole-cells into the posterior depression of the ventral groove.

FIG. 30. Lateral view of same egg as Fig. 29. a, cephalic lobe of germ-band; b, invagination which will give rise to the stomodeum.

FIG. 31. View of posterior end of same egg as Fig. 29.

FIG. 32. Ventral view (Stage F). Pole-cells, partly covered by lateral folds, lie in the posterior depression of ventral groove (a). This depression is now the posterior amniotic cavity.

(*Continued on next page*)

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FIG. 33. Sagittal section through an egg similar to that shown in Fig. 32 (Stage F). The relations of pole-cells, pole-cell canal and pseudoblastodermic nuclei are here well illustrated. a, invagination which will give rise to stomodeum. × 60.

FIG. 34. Transverse section through posterior depression in ventral groove, Fig. 32 (Stage F). a, flask-shaped depression in ventral groove. × 105.

FIG. 35. Transverse section near the end of the tail-fold of an embryo of Leptinotarsa similar to Stage H, position 35. The pole-cells are creeping from the posterior amniotic cavity through the pole-cell canal. × 140.

FIG. 36. Sagittal section through the posterior end of an egg of Leptinotarsa like Stage H, position 36. One pole-cell is part way through the pole-cell canal. × 105.

FIG. 37. Sagittal section through the tail-fold of an embryo in Stage J, position 37. Mesoderm has appeared. A few pseudoblastodermic nuclei show signs of disintegration. × 105.

FIG. 38. Transverse section of tail-fold of embryo in Stage J, (38), showing passage of pole-cells through pole-cell canal. a, flask-shaped depression in ventral groove. × 105.

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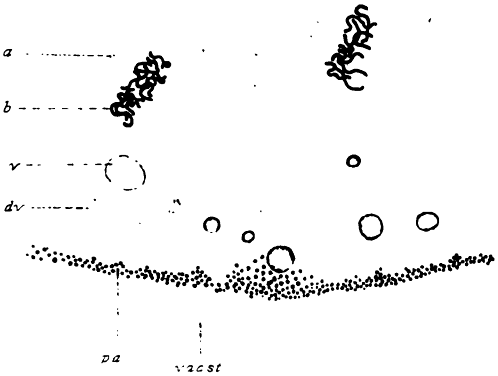

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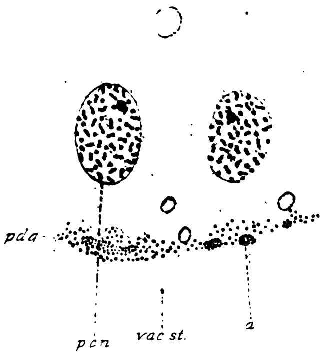

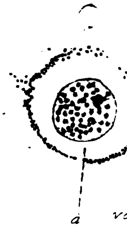

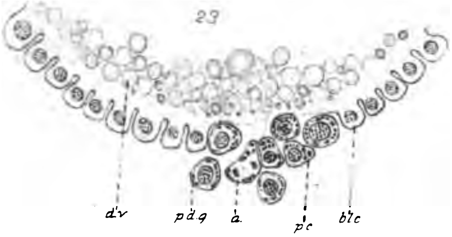

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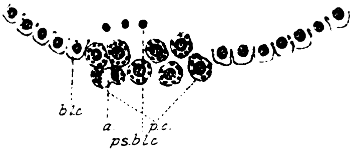

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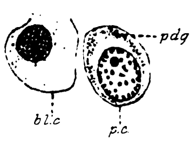

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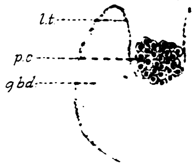

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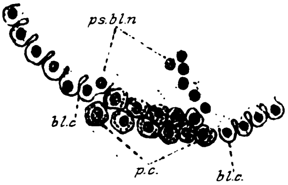

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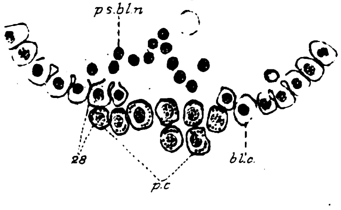

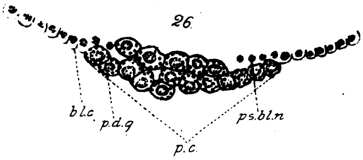

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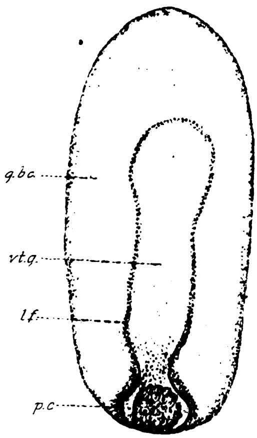

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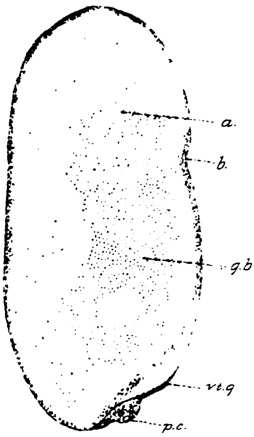

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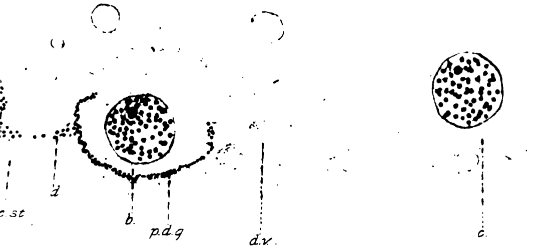

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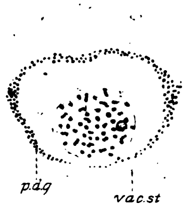

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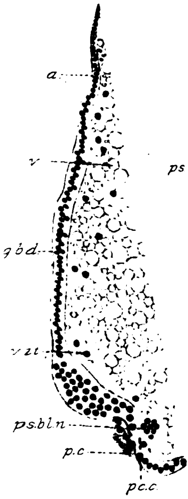

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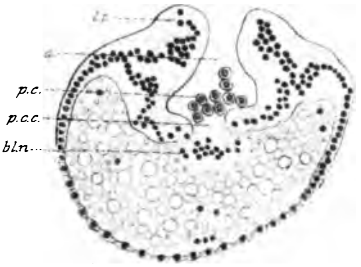

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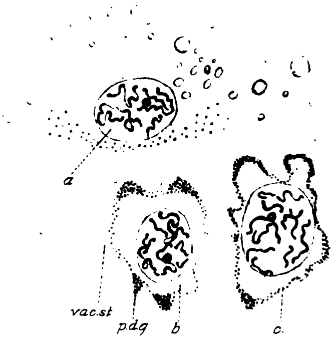

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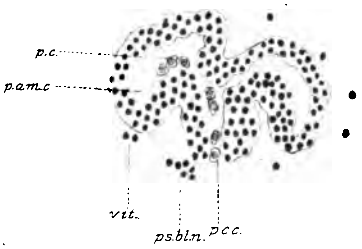

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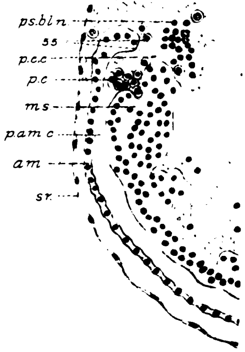

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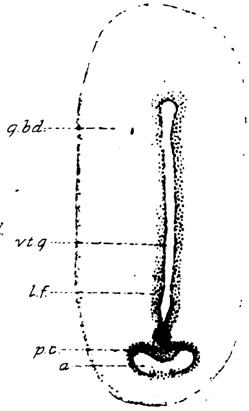

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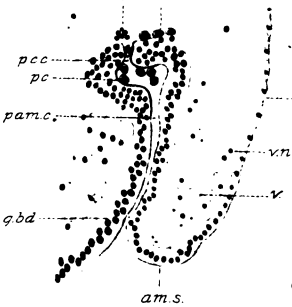

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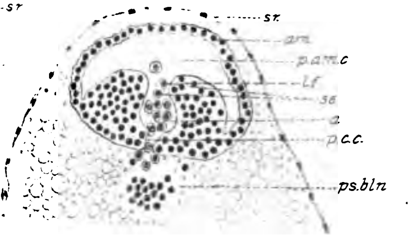

## EXPLANATION OF PLATE IV.

FIGS. 39 to 45. Sagittal sections through the tail-fold or posterior end of embryos, showing the migration of the germ-cells within the body, and their crowding together to form the germ-glands. Figs. 39 and 42, × 105; the others, × 140.

FIG. 39. Embryo to Stage K (39). Almost all of the pole-cells have penetrated into the embryo.

FIG. 40. Embryo in Stage L (40). a, two pole-cells lying in the much shortened pole-cell canal.

FIG. 41. Embryo in Stage M (41). The germ-cells are closer together than in a younger embryo (Fig. 40).

FIG. 42. Embryo in Stage N (42). The germ-cells form a distinct germ-gland with epithelium of mesoderm-cells.

FIG. 43. Embryo in Stage O (43). One of the germ-glands lies at the side of the median line near ventral surface of embryo.

FIG. 44. Embryo of 86 hours slightly older than Stage O. Germ-gland has been carried near dorsal surface by lateral growth of embryo around the yolk.

FIG. 45. Embryo of 105 hours (male). The germ-gland has become constricted, forming a dumb-bell-shaped structure.

FIG. 46. Sagittal section through germ-gland (female) of an embryo 105 hours old, showing the developing terminal filaments. × 320.

FIGS. 47 to 53. Transverse sections through the tail-fold or posterior end of embryos, showing the separation of the germ-cells into two germ-glands which are carried near the dorsal surface by the lateral growth of the embryo around the yolk. Fig. 47, × 105; all the others, × 140.

FIG. 47. Embryo in Stage K (47). Part of the pole-cells are still on their way through the pole-cell canal. (For sagittal section of this stage see Fig. 39.)

FIG. 48. Embryo in Stage L (48). Three pole-cells on each side of the tail-fold. Sagittal section in Fig. 40.

FIG. 49. Embryo in Stage M (49). Germ-cells lie close to coelomic cavity on either side of tail-fold. Sagittal section in Fig. 41.

FIG. 50. Embryo slightly older than Stage M. Germ-cells have acquired an epithelium of mesoderm-cells.

FIG. 51. Embryo in Stage O. Germ-gland on one side of median ventral line. Sagittal section in Fig. 43.

FIG. 52. Embryo 86 hours old. Germ-gland has been carried near dorsal surface. Sagittal section in Fig. 44.

FIG. 53. Embryo 105 hours old, showing germ-gland (female) near median dorsal line. The terminal filaments of the ovary are developing.

FIG. 54. Frontal section of an embryo in Stage O. × 140.

FIG. 55. A pole-cell and adjacent ectoderm-cell enlarged from Fig. 37 (55). × 850.

FIG. 56. Two pole-cells showing amoeboid processes, enlarged from Fig. 38 (56). × 850.

FIG. 57. Two pole-cells (a), two mesoderm-cells (b), and two ectoderm-cells (c), enlarged from Fig. 41 (57). × 850.

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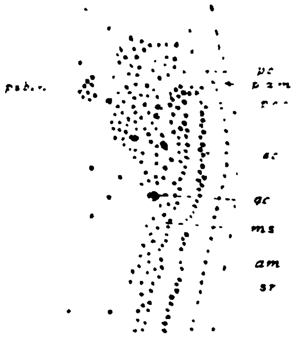

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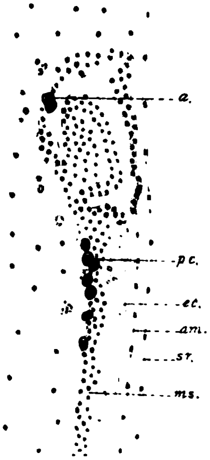

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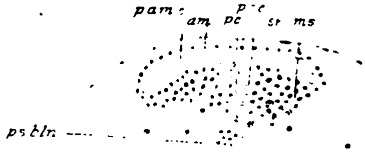

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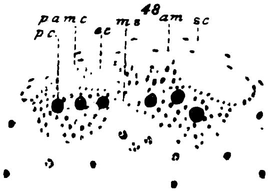

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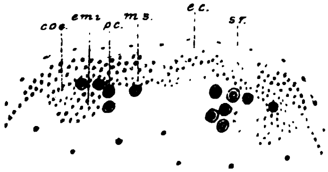

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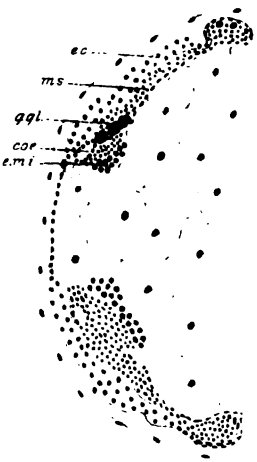

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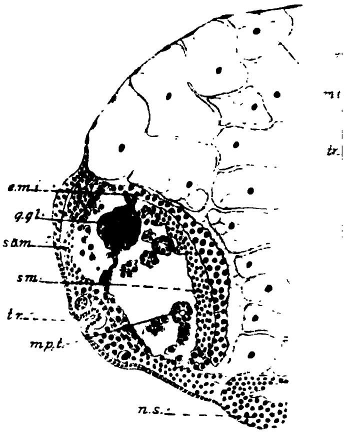

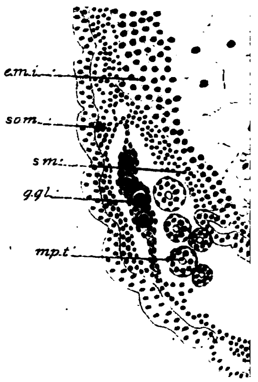

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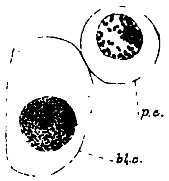

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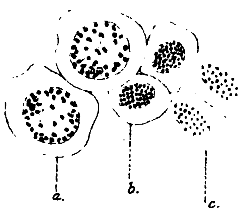

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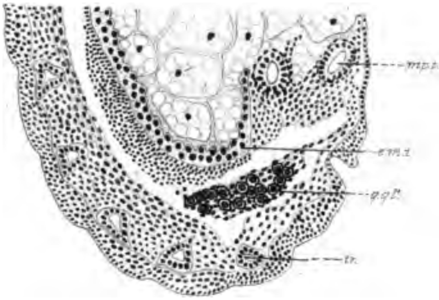

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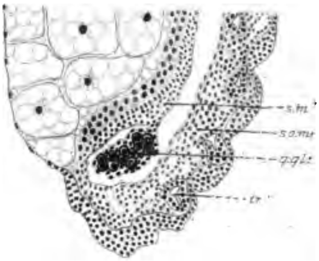

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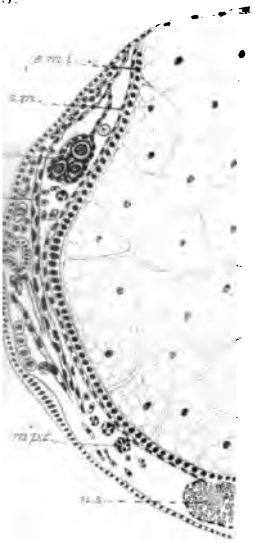

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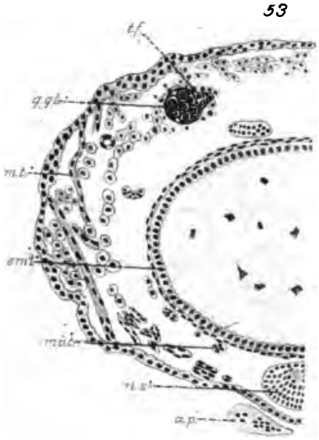

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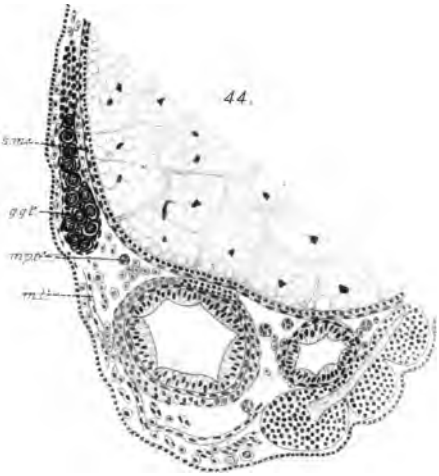

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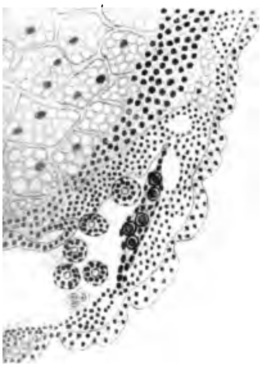

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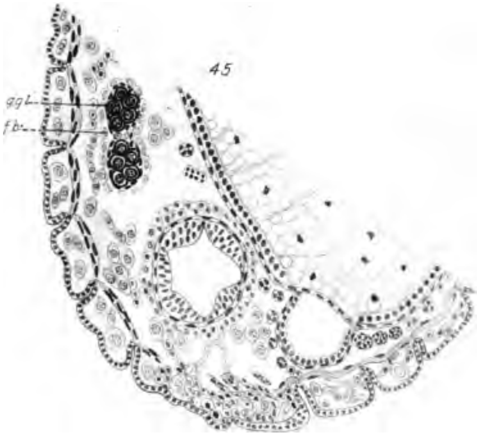

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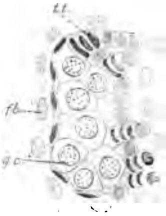

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